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Introduction to..

AN INTRODUCTION TO
PLANT BIOCHEMISTRY

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BY

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'So it will not, I presume, be an unacceptable entertainment, . . . but will rather add to the pleasure, with which vegetable Nature in her prime verdure charms us: To see the steps she takes in her productions, and the wonderful power she therein exerts: The admirable provision she has made for them, not only vigorously to draw to great heights plenty of nourishment from the earth; but also more sublimed and exalted food from the air, that wonderful fluid, which is of such importance to the life of Vegetables and Animals: And which by infinite combinations with natural bodies, produces innumerable surprizing effects; many instances of which I have here produced.'

From the Dedication to H.R.H. GEORGE, PRINCE OF WALES,
in *Vegetable Staticks*, by Stephen Hales, B.D., F.R.S. (1727)

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Introduction to

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PREFACE

The aim of the present book is to provide students of Botany with an introductory account of the chemical nature and relationships of the substances elaborated by plants.

The text is divided into parts corresponding to the large groups of the fats, carbohydrates, proteins, etc. While the last section has been reserved for a general treatment of the inter-relationships of the various compounds in such phenomena as photosynthesis, respiration, germination, and fruit ripening, the fundamental metabolism of the higher plants has been kept in view throughout. Stress has also been laid on the distribution of the various substances in plants, and on the striking relationships between chemical structure and botanical classification, which are only now becoming widely recognised.

Too often the student of Botany finds his preparation for Plant Biochemistry in a short course of organic chemistry, which is insufficient to enable him to cope with the complex chemical structures and reactions taking place in the plant. For this reason, and to make the book useful to students who have had no training in organic chemistry, the subject has been developed logically from the start according to the modern theories of organic structure. The usual sequence of compounds has been modified in a few instances, *e.g.* in grouping the aldehydes with the carbohydrates rather than between the alcohols and acids, in order to keep in juxtaposition substances intimately connected in the metabolic processes of the higher plants. The biochemistry of lower plant forms is not dealt with in detail, and is only incorporated in places for purposes of comparison.

The material is an elaboration of a series of about thirty lectures given to students in biochemistry, and the accompanying experiments form a parallel elementary course of practical instruction. None of the experiments requires elaborate apparatus, and extractions which necessitate hours of labour have been avoided. A few quantitative experiments have been incorporated in the scheme, as it has always seemed undesirable to the writer to dissociate qualitative from quantitative investigations in the field of biochemistry, even though some of the latter determinations admit of errors which can only be eliminated by the application of an elaborate technique and the use of research methods of long duration which are unsuitable for teaching purposes.

Many books, reports, and articles in scientific periodicals have been consulted in the compilation of this book. A bibliography for each part, for the use of the student, has been incorporated at the end of the text; this includes standard works, monographs, and papers, written mainly in English, and giving good accounts of the subject in question. In addition, the following texts have been widely consulted: Read, *A Text-Book of Organic Chemistry* (G. Bell & Sons); Plimmer, *Practical Organic and Biochemistry*; Czapek, *Biochemie der Pflanzen*; the *Annual Reports on the Progress of Chemistry* (Chemical Society); Pryde, *Recent Advances in Biochemistry*, 3rd ed.; Onslow, *Principles of Plant Biochemistry*, Part I; Onslow, *Practical Plant Biochemistry*, 3rd ed.; Haas and Hill, *The Chemistry of Plant Products*, vols. i and ii, 4th ed.; Barton-Wright, *Recent Advances in Plant Physiology*; Maximov (trans.), *Text-Book of Plant Physiology*.

By kind permission of the author, the illustrations for figs. 3, 5, and 6 are taken from Professor Read's *Introduction to Organic Chemistry* (G. Bell & Sons), and figs. 1 and 7 from the same author's *Text-Book of Organic Chemistry*. Fig. 4 is taken from Dr Fritch and Dr Salisbury's *An Introduction to the Structure and Reproduction of Plants* (G. Bell & Sons), by kind permission.

I am deeply indebted to Dr E. Ashby for the care with which he has read the entire manuscript, and for many valuable suggestions which have been incorporated in the text.

I have also to thank Professor John Read for his encouragement, criticisms, and editorial help.

C. C. S.

SWANLEY
February 1934

PREFACE TO THE SECOND EDITION

IN this edition the whole book has been carefully revised and brought up to date. The recent elucidation of the chemical nature of certain enzymes has necessitated additions to several sections, and the chapters on enzymes, photosynthesis, nitrogen metabolism, and respiration have been largely rewritten, and rearranged to form a logical development of the new theories.

C. C. S.

HUNTINGDON VALLEY
Penna., U.S.A.
October 1948

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PART I. INTRODUCTION

CHAPTER I

THE CHEMICAL COMPOSITION OF PLANTS

Introduction

ALL plants are built up of **cells,** each composed of a **nucleus** embedded in **protoplasm,** which in turn is enclosed by the **cell-wall.** All except young cells and adult active cells are *vacuolated*—that is, they contain spaces in the protoplasm filled with an aqueous liquid having substances in solution and in suspension; this liquid is called the **cell-sap.** In the higher plants, cells are organised into tissues with different functions, *e.g.* root, leaves, stem. In addition, dead cells may accumulate, giving permanent tissue still retained by the living plant, *e.g.* woody tissue. The materials of which all cells, both plant and animal, are composed contain large amounts of the element **carbon,** also hydrogen, oxygen, nitrogen, sulphur, and other elements, and are classed as **organic compounds.** When a plant or animal tissue is burned in air, these organic compounds are oxidised by atmospheric oxygen to gaseous products including carbon dioxide and water, and there remains a small amount of a white **ash.** It was early recognised that this incombustible residue was composed of materials which also occurred in, or could be prepared from, rocks and the soil, *e.g.* potassium carbonate and calcium oxide, and the chemical composition of these substances was gradually elucidated. The combustible or organic part, on the other hand, was essentially the product of living organisms, and until the beginning of last century such organic compounds were regarded as the product of a vague influence called 'vital force.' Although some individual compounds had been isolated in a pure state, *e.g.* citric acid, and their properties and inter-relationships discovered, it was considered impossible to prepare them from inorganic or non-living matter. This *vitalistic theory* was disproved in 1828 by Wöhler's synthesis of urea, $CO(NH_2)_2$, a typical product of animal metabolism, by an intramolecular rearrangement of the inorganic compound ammonium cyanate, $(NH_4)CNO$, on warming. The synthesis of ethyl alcohol followed in the same year, and a large number of plant and animal products have now been synthesised in the laboratory. The term *organic compound* is therefore now applied to *compounds of carbon*, only some of which are elaborated by plants.

Expt. 1. *Determination of the Dry Weight and Ash in Plant Tissue*

1. Weigh out accurately between 2 and 3 grm. of plant material into an evaporating basin, and dry in the air oven at 100° C. to constant weight. Calculate the percentage dry weight.

2. Remove the residue from (1) carefully into a crucible, ignite gently over a Bunsen flame for 5 minutes, then place the crucible in a muffle furnace and heat until constant weight is obtained. Calculate the percentages of ash in both the fresh and the dry weights.

Expt. 2. *Examination of Organic Substances for the Constituent Elements*

Use (*a*) pure compounds such as sucrose and urea; (*b*) plant products such as Wheat flour, Pea flour; and (*c*) plant tissues such as leaves, roots, etc.

1. *Test for Carbon and Hydrogen.* Mix some of the material with about ten times its volume of copper oxide and place in a hard-glass test-tube held horizontally by a clamp. Place a little anhydrous copper sulphate near the neck of the tube, and fit the tube with a cork carrying a delivery tube dipping into lime-water. Heat the mixture strongly. Note the presence of water formed by the oxidation of the *hydrogen* in the substance (the copper sulphate turns blue), and the presence of carbon dioxide from the *carbon* present (the lime-water turns milky).

2. *Soda-Lime Test for Nitrogen.* Mix some of the substance with soda-lime in a dry test-tube, heat strongly, and test the evolved gases for ammonia by smell and action on litmus paper. (This test is given by ammonium, amino-, and amide derivatives, alkaloids and proteins.)

3. *Lassaigne's Test for Nitrogen, Halogens, and Sulphur.* Place a small pellet of clean sodium and a little of the substance in an ignition tube, and, holding it with crucible tongs, heat it in the Bunsen flame until all reaction has ceased. Plunge the red-hot tube into an evaporating dish containing about 20 c.c. of distilled water. Boil well to extract all soluble material, filter, and divide the filtrate into three portions.

(i) To one portion add a few c.c. of a saturated solution of ferrous sulphate, then boil. Cool, add a few drops of ferric chloride solution, and acidify with dilute hydrochloric acid. A blue solution or precipitate shows the formation of ferric ferro-cyanide (Prussian blue) and therefore the presence of *nitrogen* in the original substance.

(ii) Acidify the second portion with dilute nitric acid, boil (especially if nitrogen is shown to be present in (i)), and add silver nitrate solution. A precipitate shows the presence of a *halogen* acid as a silver salt. Identify chloride, bromide, or iodide by the usual inorganic tests.

(iii) To the third portion add a few drops of sodium nitro-prusside solution. A violet coloration shows the presence of *sulphur* in the original substance.

4. *Test for Sulphur and Phosphorus, using Fusion Mixture.* Heat some fusion mixture (potassium nitrate and sodium carbonate) slowly in a crucible until it fuses. Allow it to cool slightly, then add a very little of the substance to be tested. Heat the mixture gently until all signs of the substance disappear. Repeat this twice. Then allow the contents of the crucible to cool somewhat, dissolve them in hot water and divide the solution into three portions.

(i) Acidify with hydrochloric acid, and add barium chloride solution. A white precipitate of barium sulphate, insoluble on boiling, indicates *sulphur* in the original material.

(ii) Add a little ammonium hydroxide, then acidify with dilute nitric acid, and boil with excess ammonium molybdate solution. A white or yellow precipitate of ammonium phosphomolybdate indicates *phosphorus.*

(iii) Add excess ammonium hydroxide, then magnesia mixture. A white precipitate of magnesium ammonium phosphate confirms the presence of *phosphorus.*

Classification of Plant Materials

It is possible to determine what organic substances are present in each plant tissue, and in some cases in each part of the cell. For instance, the cell-wall is composed of **cellulose** and other compounds belonging mostly to the carbohydrate group, the protoplasm is composed mostly of **protein,** the cell nucleus of **nucleoproteins** and **lipins,** while the cell-sap contains in solution simple **carbohydrates, soluble nitrogenous compounds, acids,** and other organic substances, in addition to **inorganic ions,** such as potassium, calcium, nitrate, and phosphate. Some compounds occur as *crystalline deposits, e.g.* calcium oxalate, others as *granular bodies, e.g.* starch and the protein 'aleurone grains.' Some water-insoluble compounds, such as the fats and the 'essential' or volatile oils, do not occur as definite organised bodies, though the former are deposited in the desiccated vacuoles of many ripe seeds. Many of these substances can be isolated unchanged from the plant, *e.g.* the cell-wall and storage materials, but the protoplasm cannot be isolated from the cell unchanged, the rupturing of the cell-wall bringing about irreversible changes in the protoplasm, to which the term 'death' is applied.

This distinction of occurrence of these various substances in different parts of the plant is also to some extent a distinction of **chemical structure** and of **function** in the plant. The plant is a living organism in which compounds are being built up, broken down, and stored in seeds for the use of the embryo of the next generation of plants. Thus the plant must be regarded as a chemical factory, and the inter-relationships of the various compounds must be known before a complete picture can be obtained of the changes that are included in the term 'plant metabolism.' Hence we shall classify and study these chemical compounds occurring in plants according to their chemical structure, and then discuss their relationships in plant metabolism.

1. **Fats and Oils.**—These contain carbon, hydrogen, and oxygen, and are insoluble in water, but soluble in ether and chloroform. They are the chief storage material of seeds (p. 34). Certain other substances are extracted with the fats, *viz.* waxes, lipins, and sterols. Most of these compounds are esters, being built up from acids and alcohols. All these types are discussed in Part II.

2. **Carbohydrates.** These compounds of carbon, hydrogen, and oxygen include the **sugars,** formed in photosynthesis, occurring as storage products, and used up in respiration; the **starches,** which are also storage products; and the **celluloses,** which form the framework of the plant. The sugars are related in chemical structure to other simple substances, the aldehydes and ketones, which are probably also metabolic derivatives of sugars in the plant. These are all discussed in Part III.

3. **Plant Acids.** Other relatively simple compounds of carbon, hydrogen, and oxygen possess acidic properties. They occur in plants in solution, and as salts with bases both in solution and as crystalline deposits. These form Part IV.

4. **Proteins.** The compounds of nitrogen in the plant include the proteins, which comprise the protoplasm and function also as storage materials in seeds. The amino-acids and amides are simpler related substances. These are all discussed in Part V.

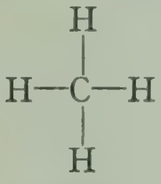
5. Other compounds occurring in plants are the **tannins,** which give the astringent taste to some unripe fruits; the **pigments;** the **essential oils,** which give plants their distinctive odours; and the **alkaloids,** which occur as toxic constituents of such plants as the Deadly Nightshade. These are all characterised chemically by containing cyclic structures (p. 5), and are discussed in Part VI.

A comparison of the various chemical compounds in different plants with the botanical classification of the latter leads to the interesting result that *many genera and even some natural families are characterised by certain chemical compounds.* Examples will be found in the acid components of the molecules of the seed fats which are characteristic of certain families, *e.g.* petroselinic acid of the *Umbelliferæ*, and erucic acid of the *Cruciferæ*. Similarly, certain glycosides are characteristic of botanically related plants, while quite distinct proteins are built up in the seeds of the *Leguminosæ* and the *Gramineæ*. On the other hand, even where species are almost indistinguishable morphologically, their chemical composition may be so entirely different in one or more types of compounds that a real species difference is indicated. The chemical constituents of the essential oils were utilised in the classification of *Eucalyptus* and other Australian plants, and of the genera *Cymbopogon* and *Andropogon* of the Indian grasses. Also many closely related species are distinguished by the alkaloids they contain, *e.g.* species of both *Datura* and *Hyoscyamus* of the *Solanaceæ.*

Characteristics of Organic Compounds

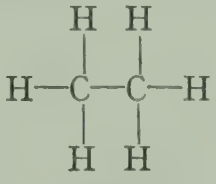
Classification. It has been shown that organic compounds, or compounds of carbon, are readily burnt in air to carbon dioxide and water, while inorganic substances with a few exceptions do not burn.

Another fundamental difference between organic and inorganic compounds is illustrated by the fact that there is only one compound of the elements sodium and chlorine, *viz.* common salt, and only three or four compounds of the elements sodium, chlorine, and oxygen, *viz.* sodium hypochlorite, NaClO, sodium chlorate, $NaClO_3$, etc. On the other hand, there are thousands of compounds of carbon and hydrogen alone, and still larger numbers of compounds containing carbon, hydrogen, and oxygen. The difference exists because the carbon atom possesses the peculiar property of combining with other carbon atoms to give **chains of carbon atoms.** Compounds containing an open-chain structure are termed **aliphatic,** and the simplest members are methane, or marsh gas, CH_4, and ethane, C_2H_6. Other compounds contain a ringed or **cyclic** structure; the most common are those derived from benzene, C_6H_6, which contains a six-membered carbon ring, and these are



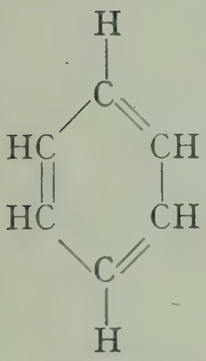
$$\begin{array}{c} H \\ | \\ H-C-H \\ | \\ H \end{array}$$

Methane

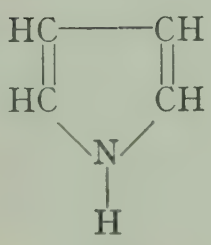


$$\begin{array}{c} H\ \ H \\ |\ \ \ | \\ H-C-C-H \\ |\ \ \ | \\ H\ \ H \end{array}$$

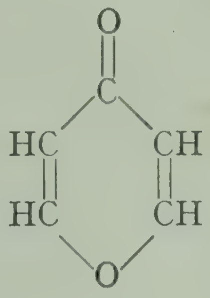
Ethane



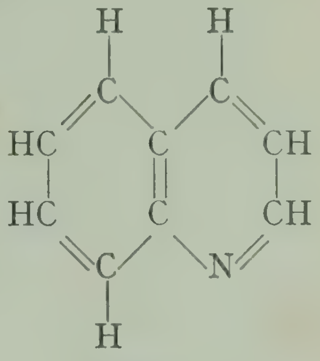
Benzene



Pyrrole



Pyrone



Quinoline

called **aromatic compounds.** Others contain a six-carbon ring structure, but resemble the aliphatic compounds in property and therefore form the **alicyclic** group. In still others, the ring structure is built up of other elements as well as carbon, especially nitrogen and oxygen, and these are classed as **heterocyclic compounds.** Six-membered rings are again the most common, *e.g. pyrone,* which is present in some of the plant pigments, but five- and four-membered rings also occur, *e.g. pyrrole* in the alkaloids and the chlorophyll molecule. Finally, fused or **condensed rings** are also known, *e.g.* the *quinoline* ring in the alkaloids.

Isomerism. It is therefore possible for many different substances to have the same percentage composition, *e.g.* the formula C_2H_6O represents both ethyl alcohol and dimethyl ether. Such substances are called **isomers,** and **isomerism** may be defined as the existence of more than one compound having the same molecular formula. The two compounds mentioned above differ in containing distinct groupings in the molecule; ethyl alcohol, C_2H_5OH, contains the alcoholic (OH) group, while dimethyl ether contains an oxygen between two carbon atoms, $CH_3—O—CH_3$. More subtle types of isomerism exist; **stereoisomerism,** for instance, depends on the different arrangements in space of the constituent groups of a molecule. The most common form of stereoisomerism met with in natural products is **optical isomerism.** The carbon atom is quadrivalent, and may be pictured as occupying the centre of a tetrahedron, the valencies being directed from the centre to the four solid angles of the tetrahedron (fig. 1).

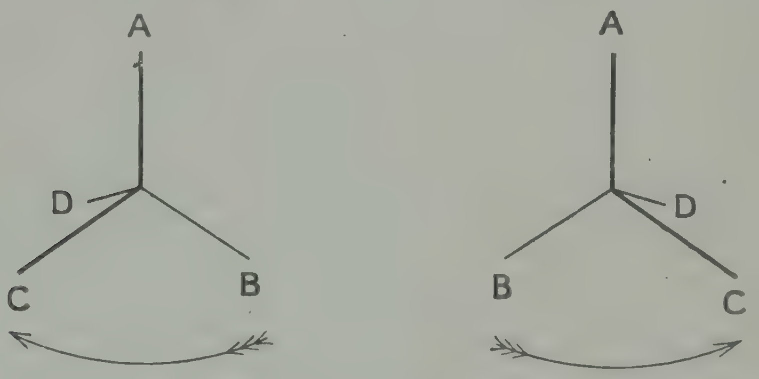


FIG. 1. Tetrahedral Configuration of Optical Isomers

If these four valencies are satisfied by four **different** atoms or groups, then two such tetrahedra exist, one being the non-coincident *mirror image* of the other. Such a carbon atom is described as **asymmetric,** and the molecule itself is an asymmetric molecule. The two mirror-image forms are isomeric, and differ from each other in a striking physical characteristic, namely, each has the power of turning the plane of *polarised light*[1] through a definite angle. One turns it to the right, and is therefore *dextro*-rotatory; the other turns it equally to the left, and is therefore described as *lævo*-rotatory. These are **optical isomers,** or **optical enantiomorphs.** Each optically active substance has a distinctive **specific rotation** for a given wave-length of light, designated for sodium light by $[\alpha_D]$. A third form, namely, the equimolecular mixture of the two active forms, is called a **racemic** or externally compensated form; it is optically inactive, has the same chemical properties as the other forms, but has usually a different melting-point and solubility. One of the simplest compounds showing stereoisomerism is lactic acid. The racemic form, *dl*-lactic acid, occurs in sour milk, whereas *d*-lactic acid occurs in muscle. When more than one asymmetric centre occurs in the molecule, several stereoisomers are possible, *e.g.* tartaric acid has four forms due to two asymmetric carbon atoms, while the simple sugars contain four asymmetric carbon atoms, and five when combined with other molecules as in the glycosides. In a laboratory synthesis the *dl*-form is always obtained if all the starting materials are optically inactive, although it can be **resolved** into the two optically active forms, *e.g. dl*-lactic acid by formation of a salt with an optically active base, or of an ester with an optically active alcohol, followed by fractional crystallisation of the products.

In nature, optically active compounds are widely distributed, and often one of the stereoisomers is unknown. The proteins are all optically active, so are the sugars, the alkaloids, and many of the essential oils. Glucose always occurs in the *dextro*-form, and *l*-glucose is unknown in nature. The other chief distinction between optical isomers is a physiological one; some sugars are more readily attacked by fungi than others, while the naturally occurring optical isomer of some alkaloids is more toxic than the enantiomorphous form. Many explanations of this asymmetry in nature have been advanced.[2] *Circularly* polarised light occurs at the surface of the sea, and has been credited with the inauguration of optically active forms. Certainly, once a predominance of one enantiomorphous form over another has been achieved, the formation of a large number of other optically active substances is possible through ordinary chemical reactions (asymmetric synthesis). The elaboration by the plant, even in photosynthesis, of optically active substances indicates that the minutest selectivity is displayed by the organised structure of the plant between such closely related compounds as optical isomers.

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[1] Polarised light is light made to vibrate in one plane by means of a Nicol prism. For a more detailed treatment of stereochemistry see Read, *A Text-Book of Organic Chemistry*; or Read, *An Introduction to Organic Chemistry*.

[2] See Mitchell, *The Cotton Effect*, 1933, p. 74 (G. Bell & Sons).

Homologous Series. The study of organic compounds is simplified by the occurrence of homologous series, in which the formula of a compound differs from that of the preceding and succeeding members of the series by a constant term (CH_2). An example taken from the aliphatic compounds is the **Paraffin Series** of hydrocarbons. Ethane, C_2H_6, is derived from methane, CH_4, by the substitution of a hydrogen atom by a CH_3 group, or by the nett addition of CH_2. Similarly, the next higher **homologue** is propane, C_3H_8, and the **general formula** for the paraffin series is C_nH_{2n+2}. Parallel homologous series are furnished by the aliphatic alcohols (Chap. III), the fatty acids (Chap. IV), the aldehydes (Chap. VII), the aromatic hydrocarbons, benzene, C_6H_6, toluene, $C_6H_5{\cdot}CH_3$, etc., and the aromatic aldehydes and acids (Chap. XVIII). Members of homologous series have in general *similar chemical properties*, but show a gradation in physical properties such as melting-point, boiling-point, and solubility. This is clearly seen in the fatty acids (Chap. IV).

Plant Metabolism

The classical experiment of van Helmont (1577–1644) in which he grew a Willow for five years in soil watered with rain-water, and found that it gained 164 pounds in weight, while the soil lost only 2 ounces, was taken as proof that **water** was the sole 'principle' from which plants build up their structure. Other investigators of the same period showed that **salts** were drawn from the soil. John Woodward (1699) found that the growth of Spearmint increased with the impurity of the water in which it was grown, and concluded that 'we may reasonably infer that **earth,** and not water, is the matter that constitutes vegetables.' It was then thought that all the carbonaceous matter in plants was derived from the humus in the soil. Although some investigators, such as Stephen Hales (1727), thought that plants in some way used **air,** it was not till long after Priestley and Ingen-Housz had discovered the 'breathing' of plants that this 'humus theory' was finally quashed by the thundering polemics of Liebig. The source of plant nitrogen was elucidated still later, the delay being due to some investigators using leguminous plants and others not, and to the failure to recognise the part played by the nodule bacteria in the fixation of atmospheric nitrogen by the former class.

The term **metabolism** includes all the vital processes in plants, both **anabolism**—that is, reactions in which new tissue is built up with the absorption and storage of energy—and **katabolism,** in which substances undergo chemical changes with the liberation and utilisation of that stored energy. The great anabolic process in plants is **photosynthesis.** Carbon dioxide is absorbed by the chloroplasts; and the green pigment of these plastids, chlorophyll, absorbs light energy which converts the carbon dioxide in the presence of water into carbohydrates. From these are synthesised all the other carbon-containing compounds in the plant, with the addition of ions such as nitrate, sulphate, phosphate, potassium, etc., absorbed from the soil by the roots and carried in the transpiration stream. In addition to this formation of new tissue, the plant lays up stores of food (or energy-containing compounds) in seeds, roots, and tubers, and this involves in most cases the **translocation** of relatively simple soluble substances to the tissue in question, and the laying down there of the storage material by synthesis. Finally, the plant requires energy to grow, and material is broken down chemically with the liberation of energy in the process known as **respiration.** The chemical transformations concerned in these processes in the life of a plant are discussed in Part VII. The mechanism is not always clear, for there is often no parallel between the transformation in the laboratory at high temperatures and with drastic reagents, and in the plant organism at ordinary temperatures. One of the main factors in such reactions is the part played in the plant by **enzymes.** These are organic catalysts elaborated by the living organism, but able to act independently of the living cell. Through the agency of enzymes, complex insoluble substances are readily broken down into soluble compounds which can be translocated. Further, in some cases, if not all, these enzymes are able in the cell, under appropriate conditions, to catalyse the reverse process of the building up of complex materials from simple components. One type of chemical reaction catalysed by a large group of enzymes is **hydrolysis,** the addition of the elements of water to a molecule, with the consequent severance of the latter into two or more simpler molecules. For instance, sucrose (cane sugar) is hydrolysed to two molecules of simpler sugars, glucose and fructose; a fat is hydrolysed to acids and glycerol; and the protein molecule is hydrolysed to amino-acids. The reverse process of **condensation,** with the elimination of one or more molecules of water, is common in the synthetic processes in plants, and may be catalysed by the same enzymes. Thus, starch is built up in Potato tubers and in cereal grain from soluble sugars translocated from their seat of synthesis in the leaves.

Animal metabolism stands in sharp contrast to plant metabolism in that in the former all energy intake and storage comes from the food ingested, and therefore is ultimately dependent on the photosynthetic process in plants. The chemical processes in the animal consist in the reorganisation of the food material for the synthesis of tissue, and in the decomposition of some of it in respiration; the energy liberated in the latter process is used for maintaining the body alive, for producing muscular movements, etc., and for maintaining the body temperature at a constant level (in the warm-blooded animals).

CHAPTER II

THE COLLOIDAL STATE

The Nature of Colloidal Solutions

THE *states* in which individual substances are present affect their physical properties, and therefore also vary their participation in chemical change, and determine to some extent their function in plant metabolism. Carbon dioxide enters the stomata of the green leaf as a *gas*, and obeys the laws of gaseous diffusion. The cellulose of the cell-wall is a fibrous *solid*, insoluble in water or in the plant cell-sap, and therefore must be built up *in situ*. The cell-sap, a *liquid* which fills the vacuoles of cells, contains in *solution* both electrolytes and non-electrolytes. Electrolytes are substances which exist as ions in solution, *e.g.* potassium chloride in aqueous solution is ionised to the potassium and the chloride ions. Non-electrolytes, on the other hand, do not ionise in solution, *e.g.* the sugars. The cell-sap also contains various substances in *suspension*. In addition, there is the protoplasm of each cell, a gelatinous mass composed of a number of substances with peculiar properties of water absorption and swelling, as is exemplified, for instance, by the absorption of water during the germination of Peas. This introduces us to another state of matter, namely, that of **colloidal solution.**

Many organic compounds isolated from plants, including starch, pectin, proteins, tannins, and the gums, appear to dissolve in water under certain conditions, giving solutions whose only obvious difference from a solution of sugar (non-electrolyte) or common salt (electrolyte) is that the former solutions may appear opalescent. Similarly, certain inorganic substances that are normally considered insoluble, such as ferric hydroxide, gold, and silver, can be obtained in solution. The solutions of both these inorganic and organic substances differ, however, from the *true* solutions of sugar and salt in several ways. Graham (1861) was the first to propose the name 'colloidal solution' for this new type and to contrast their properties. He showed that substances in true solution could diffuse through parchment membranes much more rapidly than those in colloidal solution, and that the latter could, in fact, be separated from the former by this process, which he called **dialysis.**

EXPT. 3. *Separation of Starch and Salt by Dialysis*

Make a starch solution by mixing 2 grm. of starch to a paste with cold water and pouring it into 100 c.c. of boiling water, stirring for a few minutes, and then allowing the solution to cool. Show with a little of the starch solution that it gives a blue colour with iodine solution. Make up a 2 per cent. sodium chloride solution, and show with a little of the solution that it gives a white precipitate of silver chloride on addition of silver nitrate. Now mix equal volumes of the starch and salt solutions and place in a parchmentised diffusion shell, suspended in a beaker full of distilled water. From time to time test the water in the beaker with iodine and with silver nitrate solutions. It will be found that the sodium chloride has diffused through the membrane, while the starch has not. Also, if the water in the beaker is periodically renewed, the parchment shell will be found eventually to contain only starch and no sodium chloride.

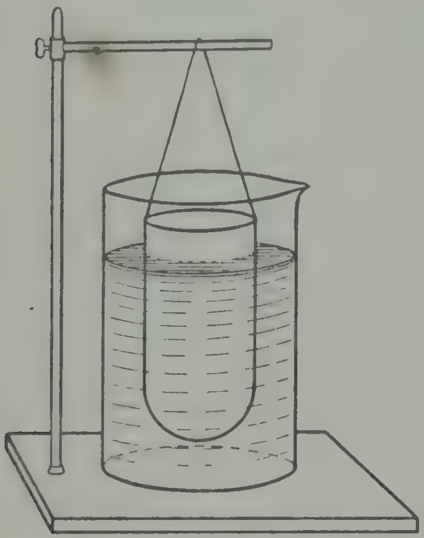

FIG. 2. Dialysis Experiment

This permeability to true solutions and not to colloidal solutions is also characteristic of plant and animal membranes, and is therefore of importance in the regulation and translocation of substances in living organisms. Starch, for instance, can never be translocated, since even in colloidal solution it cannot pass through the cell-walls.

A true solution is homogeneous or forms a single phase, whereas a colloidal solution is *heterogeneous* and consists of two phases, a *dispersed phase* and a *continuous phase.* Examples of two-phase systems are seen in suspensions, where a solid dispersed phase is in a liquid continuous phase, *e.g.* soot in water, and in emulsions, where both phases are liquid, *e.g.* olive oil in water; but suspensions and emulsions are unstable, and finally separate under the influence of gravity. Oils sometimes occur in an emulsified form in plants, forming a milky latex. Similarly, in colloidal solutions there are **suspensoids,** consisting of a solid dispersed phase in a liquid continuous phase, and **emulsoids,** consisting of a liquid dispersed phase in a liquid continuous phase. Some solids, such as starch, may form emulsoids in water, the solid dissolving in some of the water to form a concentrated solution or highly hydrated aggregate, which disperses in the remainder of the water. In both suspensoids and emulsoids, although the particles are very much smaller than in suspensions and emulsions, and cannot be seen in a microscope, the presence of two phases can be shown by the following and other methods:—

1. **The Tyndall Effect.** If a concentrated beam of light illuminates a colloidal solution and the latter is examined at right angles to the beam, a bluish cone of light is observed. This is due to the scattering of the light by the particles of the dispersed phase in the same way as a beam of sunlight entering a dark room shows up the dust particles by the light scattered from each particle. Zsigmondy used this effect to elaborate the **ultramicroscope;** a powerful beam of light is concentrated on the solution, which is then observed through a microscope set at right angles to the path of the light. In this way the discrete particles of the dispersed phase can be counted.

2. **Ultrafiltration.** The dispersed phase can be separated from the continuous phase by filtration through special filters made of collodion, and as different sizes of pores can be made, the relative sizes of the colloidal particles can be determined.

Properties of Colloidal Solutions

Colloidal solutions contain particles larger than the simple molecules or ions of true solutions; these particles may vary from solution to solution, and varying sizes may be found even in the same solution. Such particles have a very large surface compared with their volume, and hence one of the characteristics of colloidal solutions is **adsorption,** namely, the concentration of other substances on the surface of the particles.[1] Because of this adsorption, a definite arrangement of substances can occur at the interface of the phases, depending on the solubilities of the adsorbed substances in these phases. Some compounds, such as the aliphatic alcohols and acids, contain **polar groups:** the alcohol or acid group is soluble in water, while the long hydrocarbon chain (p. 27) is insoluble in water. Hence in contact with water the molecules are orientated in such a way that the polar groups are attracted to and held by the water, while the rest of the molecule stands away from it. These substances then, when present in small amounts, tend to form on water a **monomolecular film,** all the molecules standing approximately parallel (and vertical on closest packing) with only their polar groups in the water. In the cell-sap, and probably also in the protoplasm, the fatty acids will be arranged in some such fashion. When the two phases, oil and water, are present, the polar group is still attracted to the water, and the hydrocarbon part is soluble in oil, hence there is still a definite orientation of the molecules. Substances may contain several polar groups, *e.g.* the lecithins, and these will therefore be orientated according to the phases present and form a definite membrane (p. 54).

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[1] For more complete discussion of such phenomena, text-books on colloids should be consulted (see References for Part I, p. 320).

This adsorption of substances at interfaces may confer stability on an ordinary emulsion, *e.g.* egg stabilises the oil-in-water emulsion of mayonnaise, while soap stabilises the oil-in-water emulsion of various insecticidal sprays of 'mineral oil' and 'tar distillate washes' used on fruit-trees. Although a soap solution or pure sodium oleate confers stability on an oil-in-water emulsion, calcium oleate stabilises a water-in-oil emulsion, and therefore the stability of a water–oil system will depend on the proportion of the two ions present. Loeb has shown that certain marine organisms died in sodium chloride and in calcium chloride solutions when these were isotonic (*i.e.* of equal osmotic pressure) with sea-water, while in a solution containing a definite ratio of sodium and calcium the organisms lived. The necessity of a **physiological balance** of ions for plant growth will be noted later (p. 301). One of the reasons for this need is probably that only with this balance is the stability of the protoplasm, which contains water and oil (lipoids), assured.

Many colloids carry an **electric charge,** as may be shown by setting up a potential difference in a colloidal solution, when the particles of the dispersed phase will move to one of the poles (cataphoresis), or by fixing an emulsoid gel (*vide infra*) in one place, when a potential difference will cause a displacement of the continuous phase through the diaphragm (electroendosmosis). In some cases this electric charge is due to the **ionisation of the colloid** itself to form an ordinary ion and a colloidal ion. The best-known example is that of the *soaps* (McBain). Soaps are sodium and potassium salts of the higher fatty acids (p. 30), and ionise in water to give sodium or potassium ions and **ionic micelles.** The simple fatty acid ions formed by the ionisation of the soap are immediately combined in varying numbers with undissociated soap molecules, and usually also with water molecules. The large hydrated complexes or micelles thus formed are colloidal, and carry the total negative electric charge of the various component dissociated parts. The *proteins* form a still more complex case, as they can ionise both as bases and as acids—that is, they show **amphoteric properties** or are **ampholytes;** thus, various types of colloidal ions are possible, depending on the reaction of the medium (p. 151). In other cases he electric charge on the colloid is due to **adsorbed ions**, or to the referential adsorption of either hydrogen or hydroxyl ion even in ure water; in such cases removal of the ion or neutralisation of he charge results in the precipitation of the colloid. It is often ifficult to distinguish between these two types.

The **precipitation** of substances from colloidal solution is important, ot only in the isolation of plant substances such as the proteins, ut also in understanding some of the changes which accompany the illing of plants by frost (p. 317). Some types of precipitation of mulsoids are called **coagulation.** This is best exemplified by the ehaviour of some soluble proteins when heated, mixed with lcohol, or treated with alkalis, acids, and salts. Precipitation is sometimes reversible, *e.g.* the precipitation of protein with ammonium sulphate; in other cases it is irreversible, examples being the action of heat on egg-albumen, or the coagulation of the protoplasm of plants when killed by anæsthetics such as chloroform. One method of precipitation has already been indicated, namely, the neutralisation of the charge on the colloidal particle. For instance, the addition of dilute acid or of 'rennet' coagulates the casein of milk. With *electrolytes*, minute traces are usually sufficient to precipitate suspensoids, whereas larger amounts are required for emulsoids. The isolation of soap in technical practice depends on its 'salting out' with sodium chloride (p. 39), and most soluble plant proteins can be precipitated by ammonium and sodium sulphates. A complex case of precipitation occurs in the manufacture of jams and jellies, where the precipitation of the pectin requires the presence of both acid and sugar.

EXPT. 4. *Precipitation of Colloidal Solutions*

1. Take some *starch* solution prepared as in the previous experiment (p. 12), and show that the starch is precipitated (*a*) by alcohol, (*b*) by saturation of the solution with solid ammonium sulphate.

2. Prepare a *protein* solution as follows: Add 4 grm. of Wheat or Pea flour to 40 c.c. of cold distilled water, stir well, allow to stand for some time, then filter. The extract contains a colloidal solution of protein. Shake the solution and note that it froths. Saturate the solution with solid ammonium sulphate and note that the protein is precipitated. Filter off the precipitate and show that it will dissolve in distilled water (reversible precipitation). See also experiments on p. 144.

On the other hand, colloids may be **protected** from precipitation, or **peptised,** by the presence of ions, salts, non-electrolytes, or nother colloidal solution. Where two colloids are concerned, the ffect may be either protection, precipitation, or sensitisation to recipitation by other substances. Some of the effects may be explained on the basis of the electrical charges carried by the colloids, *viz.* oppositely charged colloids precipitate each other, while colloids of the same sign protect each other. But many cases cannot be explained so simply. Two colloids may also affect each other by altering the **state of aggregation** or of hydration of the particles. Tannin has long been known to sensitise both positively and negatively charged dyestuffs, but it has also been shown (p. 217) that tannin in the cell-sap affects the state of aggregation of the anthocyanin pigments and thereby alters the colour.

It is well known that starch and gelatine form 'solutions' or **emulsoid sols** only when heated, and that concentrated solutions on cooling set to a gelatinous mass, called a **gel.** The substance may either separate as a gelatinous precipitate, or the whole mass may set to form a jelly. This latter phenomenon is shown by fruit jellies, owing to the presence of a colloidal solution of pectin; also by blanc-mange, owing to the presence of starch ('cornflour' from Maize), and by gelatine jellies. These jellies may contain over 90 per cent. of liquid, and yet possess a relatively rigid framework. Moreover, when dried they will absorb water and swell, and if this swelling takes place in an enclosed space, large pressures are developed. The structure of such gels is, in fact, a reversal of phase from that of the sol. An emulsoid sol of, say, gelatine and water consists of a dispersed phase of a concentrated aqueous solution of gelatine in a continuous phase of a weak aqueous solution of gelatine. The corresponding gel consists of a framework or continuous phase of the concentrated aqueous solution and a dispersed phase of the weak aqueous solution of gelatine. In some gels this framework appears to form a definite pattern like crystals or fibres, but in most cases its nature is not completely known.

EXPT. 5. *Preparation and Properties of a Gel*

Soak 1 grm. of agar-agar (p. 99) in 50 c.c. of distilled water, then boil the mixture. An opalescent solution or sol is obtained which sets to a gel on cooling. Show that this gel-formation is reversible by warming it again, and then allowing it to cool. Take a little of the warm solution in two test-tubes and show that the mucilage is precipitated (*a*) by alcohol, (*b*) by saturation with solid ammonium sulphate.

The gels discussed above are reversible, but some gels on drying cannot be made to take up water again to form a colloidal solution. The commonest example is the inorganic gel of silicic acid. Deposits of silica in plants, *e.g.* in cereals, are probably formed by the drying out of colloidal solutions of silicic acid; thus, the material known as 'tabaschir' is a porous silica gel obtained from Bamboo stems.

Electrolytes can diffuse into jellies, and if two soluble salts which form an insoluble precipitate are allowed to diffuse successively through a jelly, a banded structure is obtained (the Liesegang phenomenon). That the clear space between the bands cannot be due to supersaturation is shown by the fact that even when crystals of the reaction product are present throughout the jelly, banding still takes place. The most probable explanation is that the layer of precipitate formed adsorbs one of the components, so that the critical concentration for precipitation is not reached until the attainment of a certain distance. The banding of agate has been explained by this diffusion through, and interaction in, a silicic acid gel, and perhaps some stratified structures in the organic world, *e.g.* the stratified walls of woody fibre, are due to the same phenomenon.

To sum up, many organic substances exist in the plant in colloidal solution. The protoplasm itself is a colloidal system of several components, including proteins, the complex carbohydrates (which can exist in aqueous phase), and the fats (which belong to the non-aqueous phase). Other substances, such as phospholipins (lecithin), are intermediate, having water-soluble and fat-soluble groupings. With this variety of phases, and the phenomenon of adsorption permitting of a distribution of substances at different interfaces in different concentrations, it is possible to visualise the protoplasm as a chemical factory in which different and even opposed reactions can take place at the same time, all being nevertheless connected through the stability of the protoplasm and affected by any change in the balance of the phases. The formation of molecular films of definite polarity, composed of substances such as the lecithins, may also account in part for the selective absorption of substances by the protoplasm. The protoplasm contains about 90 per cent. of water, and is either a very viscid sol or a gel. The former seems more likely, in view of the 'streaming' movement of protoplasm which can be observed in some cells under the microscope. The viscosity of protoplasm has actually been measured.

The selectivity of the cell is shown by the action of the external layers as a **semi-permeable membrane,** and by the phenomenon of plasmolysis. A semi-permeable membrane affords passage to water much more rapidly than to ions or molecules in true solution: thus, when a solution is separated from pure water by such a membrane, the water molecules pass through the membrane into the solution and set up a pressure, known as the **osmotic pressure.** In this way a pressure gradient can be set up between cells, and water can pass from one to the other. In **plasmolysis,** a plant cell, or tissue composed of several cells, is surrounded by a concentrated solution of a substance in true solution. This solution has a higher osmotic pressure than the protoplasm, and diffusion tends to take place in such a way as to equalise these pressures. Since water diffuses more rapidly than any of the other substances present, it leaves the cell in order to dilute the outer solution; and the cell, robbed of its water, shrinks away from the cell-wall, finally contracting to a small ball.

Because of the susceptibility of colloids to change of electric charge, the preservation of the *reaction of the medium* is important in the isolation of plant substances with as little change as possible. This reaction must also be considered in any explanation of the properties of compounds which ionise in solution, especially those with amphoteric properties (p. 151), and in any discussion of the action of enzymes (p. 251). The **hydrogen-ion concentration** is the criterion of acid or alkaline reaction for all aqueous solutions: the derived **p_{H} value** is the reciprocal of the logarithm of the hydrogen-ion concentration ($p_{H} = 1|\log [H^{\cdot}]$). The hydrogen-ion concentration may be determined directly by electrometric methods, or the p_{H} value may be found colorimetrically by the use of indicators standardised by the former method.

PART II

ALCOHOLS, FATTY ACIDS, FATS AND OILS

CHAPTER III

ALCOHOLS

Occurrence. Free alcohols do not occur to any great extent in plants, but the raw material for the manufacture on a commercial scale of the simpler aliphatic alcohols is drawn from plant sources. Some of these alcohols are intermediate products in plant metabolic processes (p. 22), while other alcohols are constituents of the plant fats and waxes, and one complex alcohol is the precursor of a vitamin.

Structure and General Reactions. The **aliphatic alcohols** may be considered as derivatives of the **paraffins,** where one hydrogen atom is replaced by a **hydroxyl group** (—OH). All alcohols, aliphatic and cyclic, contain this hydroxyl group. We have seen (p. 8) that the paraffins form a homologous series; so that by this substitution we obtain a parallel **homologous series of alcohols.** Thus methane, CH_4, ethane, C_2H_6, and propane, C_3H_8, give rise respectively to *methyl alcohol*, CH_3OH, *ethyl alcohol*, C_2H_5OH, and *propyl alcohol*, C_3H_7OH. These groups, methyl, $—CH_3$, ethyl, $—C_2H_5$, etc., are often summarised under the generic term *alkyl*. Such a group is called a **radical;** that is, it passes unchanged through many chemical reactions which alter the rest of the molecule.

The presence of the hydroxyl group in alcohols gives them a resemblance in structure and chemical reactions to *water*; that is, they may equally well be regarded as derivatives of water, H—OH, in which the hydrogen atom is replaced by an alkyl group. The action of *sodium* on water is to liberate hydrogen and form sodium hydroxide; similarly, sodium acts on alcohols with the evolution of hydrogen and the formation of sodium derivatives:

$$2H{\cdot}OH + 2Na \longrightarrow 2NaOH + H_2$$
$$2R{\cdot}OH + 2Na \longrightarrow 2RONa + H_2$$

The sodium derivative of ethyl alcohol, C_2H_5ONa, is called **sodium ethoxide** or **sodium ethylate.** *Phosphorus pentachloride* reacts both with water and with alcohols, hydrogen chloride being evolved:

$$H{\cdot}OH + PCl_5 \longrightarrow HCl + POCl_3 + HCl$$
$$R{\cdot}OH + PCl_5 \longrightarrow RCl + POCl_3 + HCl$$

The new compound formed, RCl, is an **alkyl halide,** *e.g.* methyl chloride, CH_3Cl, and this evolution of hydrogen chloride from a substance on treatment with phosphorus pentachloride is a test for the presence of the hydroxyl group. The alcohols also resemble **alkalis** such as sodium hydroxide in that they form compounds with acids; such compounds are called **esters.**

$$NaOH + \underset{\text{acetic acid}}{CH_3{\cdot}COOH} \longrightarrow \underset{\text{sodium acetate}}{CH_3{\cdot}COONa} + H_2O$$

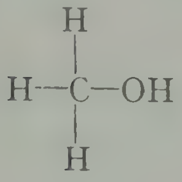
$$\underset{\text{ethyl alcohol}}{C_2H_5OH} + CH_3{\cdot}COOH \rightleftharpoons \underset{\text{ethyl acetate}}{CH_3{\cdot}COOC_2H_5} + H_2O$$

The formation of ethyl acetate from ethyl alcohol and acetic acid is a **reversible reaction.** Hence in the preparation of ethyl acetate (p. 24), concentrated sulphuric acid is added to remove the water as it is formed, thus driving the reaction in the direction of ester formation (p. 249). In the hydrolysis of ethyl acetate (p. 28), various catalysts may be employed, chief among which are dilute mineral acids or alkalis. In the former case, the free acid is obtained, while with alkali the metallic salt of the acid (*e.g.* sodium acetate) is formed instead.

When treated with concentrated sulphuric acid, alcohols undergo dehydration, and **ethers** are formed. From ethyl alcohol is obtained **diethyl ether,** a very volatile liquid, used extensively as a solvent:

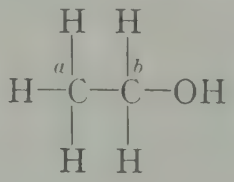
$$C_2H_5OH + HOC_2H_5 \longrightarrow C_2H_5{-}O{-}C_2H_5 + H_2O$$

Most compounds containing hydroxyl groups can form ethers with methyl and ethyl radicals, and these *methoxy* and *ethoxy* derivatives are found in many natural products, *e.g.* in the anthocyanins. It will be seen on studying the structural formula of methyl alcohol (I), that the same formula for the next higher homologue, ethyl alcohol (II), is obtained, no matter which hydrogen is replaced by $-CH_2$. But two propyl alcohols are obtained, depending on whether a hydrogen on carbon atom (*a*) or (*b*) is substituted. The first substitution gives a straight-chain alcohol, **normal propyl alcohol** (III), the second gives a branched-chain compound, *iso***propyl alcohol** (IV):



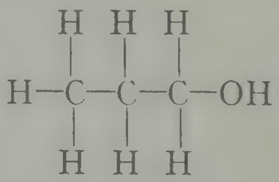
$$\begin{array}{ccccc} & & H & & \\ & & | & & \\ H & - & C & - & OH \\ & & | & & \\ & & H & & \end{array}$$

(I)



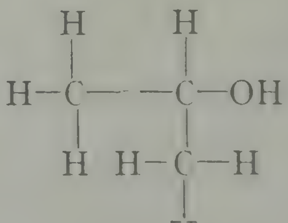
$$\begin{array}{ccccccc} & & H & & H & & \\ & & a| & & |b & & \\ H & - & C & - & C & - & OH \\ & & | & & | & & \\ & & H & & H & & \end{array}$$

(II)



$$\begin{array}{ccccccccc} & & H & & H & & H & & \\ & & | & & | & & | & & \\ H & - & C & - & C & - & C & - & OH \\ & & | & & | & & | & & \\ & & H & & H & & H & & \end{array}$$

(III)

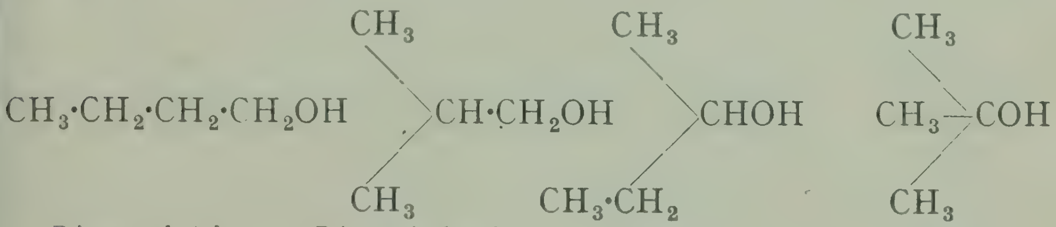


$$\begin{array}{ccccccccc} & & H & & & & H & & \\ & & | & & & & | & & \\ H & - & C & -\;- & & & C & - & OH \\ & & | & & & & | & & \\ & & H & & H & - & C & - & H \\ & & & & & & | & & \\ & & & & & & H & & \end{array}$$

(IV)

These two propyl alcohols have the same molecular formula, C_3H_7OH, but their structural formulæ, (III) and (IV), are different;

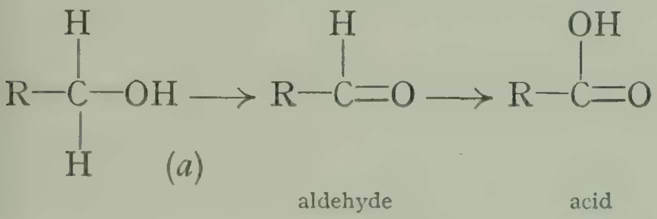
they are therefore **structural isomers.** Another difference between them is that (III) contains the group —CH_2OH, as do both methyl and ethyl alcohols, whereas (IV) contains the >CHOH grouping. We must therefore distinguish between **primary alcohols,** containing the primary alcoholic grouping —CH_2OH, and **secondary alcohols,** containing the secondary alcoholic grouping >CHOH. There are four butyl alcohols, C_4H_9OH, and one of them is a tertiary alcohol, containing the grouping ⩾COH:

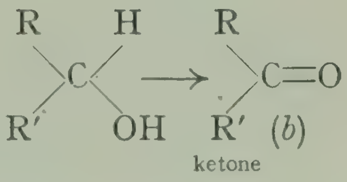


Primary *n*-butyl Primary *iso*-butyl Secondary *n*-butyl Tertiary *iso*-butyl

The **oxidation products** of the alcohols serve to distinguish these three types. A *primary* alcohol oxidises first to an **aldehyde,** then on stronger oxidation to an **acid,** both of these compounds containing the same number of carbon atoms as the original alcohol (equation (*a*)).

A *secondary* alcohol oxidises to a **ketone** (*b*), also containing the same number of carbon atoms. A *tertiary* alcohol is difficult to oxidise at all, but very vigorous oxidation gives a **mixture of acids.**





Methyl Alcohol

Methyl alcohol, or **Methanol,** CH_3OH, was first obtained by the **destructive distillation** of **wood.** The complex ligno-celluloses (p. 89) of the wood are broken down on being heated in a retort out of contact with air, and the products on cooling are a *gas,* consisting mainly of hydrogen and methane, an *aqueous liquor* called **pyroligneous acid,** which contains methyl alcohol, acetone and acetic acid, and a *tar,* while *charcoal* remains in the retort. The constituents of pyroligneous acid are separated by passing the vapour into hot milk of lime, when the acetic acid is removed as the calcium salt; the condensed vapour is then mixed with quicklime and fractionally distilled, thus separating the acetone, b.p. 57° C., and the methyl alcohol, b.p. 66°.

Methyl alcohol is now prepared synthetically on a commercial scale from water-gas and hydrogen, which combine with the aid of catalysts according to the equation: $CO + 2H_2 \longrightarrow CH_3OH$.

Methyl alcohol also occurs in the *essential oils* of many plants as **methyl esters**; for instance, methyl salicylate in oil of wintergreen (p. 232). Methyl alcohol is a colourless, poisonous liquid, which forms explosive mixtures with air, but in excess of air burns with a pale blue flame. It is miscible with water in all proportions. Methyl alcohol is important commercially as a solvent in the preparation of varnishes, lacquers, etc., as a 'denaturant' in methylated spirit, and as a starting-point in several syntheses, including that of formaldehyde (p. 59).

Ethyl Alcohol

Ethyl alcohol, or **Ethanol,** C_2H_5OH, is present in traces in many plant tissues, but can be developed in greater quantities, especially in leaves and fruits, by exposure to atmospheres deficient in oxygen. In potatoes and apples the alcoholic content is normally small, but under abnormal conditions of lack of oxygen, accumulation of alcohol and acetaldehyde may occur, due to anaerobic respiration of carbohydrate (p. 315). In apples this physiological breakdown is known as 'brown heart' or 'brown core,' and occurs particularly in some varieties, *e.g.* Newton Pippin apples. Many **ethyl esters** also occur in small amounts, especially in fruits, *e.g.* ethyl butyrate in pineapples.

Manufacture. Ethyl alcohol is prepared commercially by the **fermentation** of **sugars,** which may either be obtained directly from ripe fruits, *e.g.* grapes or apples in the manufacture of wines or cider, or the sugar is in turn derived from **starches** occurring in plant tissues, such as Maize, Barley, Potatoes, Rice (p. 83). In the latter case the starch is first hydrolysed to the sugar **glucose,** and this can be done either (*a*) by *chemical hydrolysis* with dilute mineral acid as catalyst, or (*b*) by *enzymatic hydrolysis*, when two enzymes are necessary, **diastase** from germinating Barley, which hydrolyses starch to the sugar **maltose,** and **maltase** from yeast, converting maltose to glucose. These reactions are summarised in the following equations:—

$$\text{(i)}\quad \underset{\text{starch}}{(C_6H_{10}O_5)_n} + nH_2O \xrightarrow[\text{acid}]{\text{dilute}} n\underset{\text{glucose}}{C_6H_{12}O_6}$$

$$\text{(ii)}\quad 2(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{diastase}} n\underset{\text{maltose}}{C_{12}H_{22}O_{11}}$$

$$\text{(iii)}\quad \underset{\text{maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{maltase}} 2\underset{\text{glucose}}{C_6H_{12}O_6}$$

The first part of reaction (*b*) is known in technical practice as *mashing*. Barley is moistened and allowed to germinate, then after a few days the sprouting is stopped by heating the grain in a malt-kiln. The dried ground material, known as *malt*, is mixed with the source of starch and steeped in water at 50° C. until hydrolysis is almost complete, when the enzyme is destroyed by boiling the solution (*wort*). The second stage is the production of alcohol from the glucose solution from either (*a*) or (*b*) by the enzymatic complex **zymase,** which is obtained from yeast. When method (*a*) is used, the resulting acid solution is neutralised and treated with special cultures of yeast (*Saccharomyces cerevisiæ*), upon which the liquid appears to boil, owing to the evolution of carbon dioxide. If malt is used (method (*b*)), then the second part of (*b*) and the action of zymase constitute in technical practice one process, *fermentation*. The wort from the mashing is treated with yeast, maltase acts first on the maltose, then zymase on the glucose thus formed, with the production of ethyl alcohol:

$$C_6H_{12}O_6 \xrightarrow{\text{zymase}} 2C_2H_5OH + 2CO_2$$

Other possible sources of industrial ethyl alcohol are wood and sawdust. These contain complex celluloses which can be partly converted to glucose by drastic hydrolysis with acids under pressure, and the resultant solution is fermented with yeast.

The purification of ethyl alcohol is carried out by the process of *rectification*. The dilute alcohol is fractionated in a special type of continuous-working still, the *Coffey still*, and the distillate, known as **rectified spirit,** contains 94–96 per cent. of ethyl alcohol by volume. If this is dried with quicklime and redistilled, **absolute alcohol** is obtained, containing about 1 per cent. of water. Both these forms of alcohol are used in synthetic organic chemistry and as solvents. **Methylated spirit** for industrial and laboratory use contains 95 per cent. of rectified spirit and 5 per cent. of crude methyl alcohol. **Mineralised methylated spirit** contains in addition petroleum and pyridine, and is used as a fuel and for numerous other purposes.

Ethyl alcohol can be distinguished by its ester, ethyl acetate, and by the *iodoform* reaction. The action of iodine and alkali on ethyl alcohol proceeds according to the following equation, iodoform, CHI_3, being a pale yellow crystalline solid with a distinctive odour:

$$CH_3{\cdot}CH_2OH + 4I_2 + 6NaOH \longrightarrow CHI_3 + H{\cdot}COONa + 5NaI + 5H_2O$$

Expt. 6. *Ethyl Alcohol*

1. Place five drops of alcohol in half a test-tube of water, add a few crystals of solid iodine, then sodium hydroxide solution drop by drop until the mixture is decolorised. Warm gently in a beaker of water, and note the odour and yellow crystalline precipitate of iodoform on cooling.

2. Mix small amounts of ethyl alcohol and acetic acid (or an acetate) in a test-tube, add a few drops of concentrated sulphuric acid, and note the odour of ethyl acetate on warming.

3. Warm some aqueous alcohol with a few drops of potassium dichromate solution and dilute sulphuric acid. Note the smell of acetaldehyde and the green colour of chromium sulphate.

Other Monohydric Primary Alcohols

Some of the next higher homologues of ethyl alcohol, especially the **butyl alcohols,** are produced by lower plant life, such as yeasts and bacteria, in an enzymatic decomposition similar to fermentation. Traces of *n*-**propyl,** *n*-**butyl,** and **amyl** ($C_5H_{11}OH$) **alcohols** are always found in the still-residue (*fusel oil*) from the rectification of ethyl alcohol.

Some of the alcohols from C_6 to C_{12} occur in small amounts in the *essential oils* of plants as **esters,** *e.g.* primary *n*-hexyl alcohol in the oil from the seeds, and primary *n*-octyl alcohol in the oil from the fruits of *Heracleum gigantum.*

Still higher homologues are constituents of both **plant** and **animal waxes** (p. 49), being mainly combined with acids in the form of **esters:**

Cetyl alcohol,	$C_{16}H_{33}OH$, especially in animal waxes.
Arachyl alcohol,	$C_{20}H_{41}OH$, in Raphia wax.
Ceryl alcohol,	$C_{26}H_{53}OH$, in both plant and animal waxes; Poppy wax and Carnaüba wax.
Melissyl (Myricyl) **alchohol,**	$C_{30}H_{61}OH$, in both plant and animal waxes; Carnaüba wax.

These alcohols differ from the lower members of the series in that they are insoluble in water; they are soluble in acetone and in ethyl alcohol.

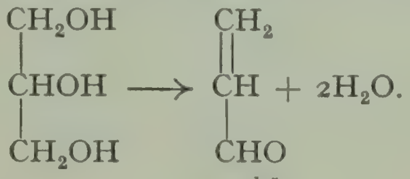
Polyhydric Alcohols.

All the alcohols so far discussed contain only one hydroxyl group, and are therefore described as **monohydric alcohols. Polyhydric alcohols,** containing more than one alcoholic group, are also known. The most important member is **glycerol,** $CH_2OH{\cdot}CHOH{\cdot}CH_2OH$, a colourless, syrupy liquid, which occurs in both plant and animal *fats* and *oils* as **esters** with acids (p. 34). It is recovered from the hydrolysis of these esters either in soap or candle manufacture (p. 39) by distilling the aqueous solution with superheated steam under reduced pressure. It has wide industrial applications in medicine, synthetic chemistry, and the manufacture of explosives.

EXPT. 7. *Glycerol*

1. Show that glycerol is soluble in water.

2. Heat some glycerol with solid potassium hydrogen sulphate in a *dry* test-tube. Note the pungent odour of acroleïn.



$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \longrightarrow \begin{array}{l} CH_2 \\ \| \\ CH \\ | \\ CHO \end{array} + 2H_2O.$$

acroleïn

3. To an aqueous solution of glycerol add copper sulphate solution, then sodium hydroxide solution. The glycerol prevents the precipitation of cupric hydroxide, and a blue solution results.

Some polyhydric alcohols closely related in structure to the sugars are found in plants. **Mannitol,** $CH_2OH{\cdot}(CHOH)_4{\cdot}CH_2OH$, a hexahydric alcohol related to mannose (p. 71), is the chief constituent of *manna*, the dried sap of a shrub, the Manna Ash (*Fraxinus ornus*). It is also present in the sap of the Larch (*Larix*); it occurs as an incrustation on some species of *Laminaria*, and in many fungi it replaces glucose. Several other hexahydric alcohols, isomers of mannitol, are elaborated by plants: **dulcitol,** related to galactose (p. 71), occurs especially among the *Scrophulariaceæ*, while **sorbitol** is widely distributed in the fruits and to a less extent in the leaves of most of the *Rosaceæ*. It also appears as a crystalline deposit on the heads of the fungus *Boletus bovinus*. **Iditol** occurs along with sorbitol in ripe Mountain Ash berries (*Pyrus Aucuparia*). **Adonitol,** $CH_2OH{\cdot}(CHOH)_3{\cdot}CH_2OH$, a pentahydric alcohol related to the pentose ribose (p. 72), occurs in *Adonis vernalis*, while **erythritol,** $CH_2OH{\cdot}(CHOH)_2{\cdot}CH_2OH$, a tetrahydric alcohol, is found in many algæ, mosses, and lichens. Two heptahydric alcohols, $CH_2OH{\cdot}(CHOH)_5{\cdot}CH_2OH$, are known, **perseitol** in the Avocado Pear (*Persea gratissima*), and **volemitol,** first isolated from the fungus *Lactarius volemus*, and later found also in the rhizomes of some species of *Primula*. An octahydric alcohol has also been found with sorbitol in the fruits of some *Rosaceæ*. This relatively wide distribution of certain alcohols, which are essentially reduction products of the sugars, points to their taking an active part in the carbohydrate metabolism of these plants, and this is borne out by experiment.

For, if detached leaves of any of the higher plants mentioned above are kept in the dark to deprive them of starch, and then floated in a solution of the alcohol which they normally contain, starch is synthesised in the leaf. Thus the *Rosaceæ* will utilise sorbitol, but not mannitol or dulcitol for starch formation, *Adonia vernalis* can only use adonitol, and the *Oleacæ* (*e.g. Fraxinus*) mannitol. These polyhydric alcohols all resemble each other in being soluble in water, and in having a sweet taste. The higher members are usually crystalline substances.

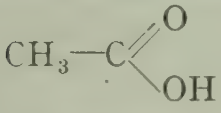
Other Types of Alcohols

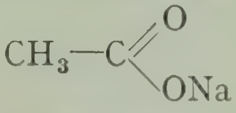
Another group of alcohols important in plant life are the **sterols**: these occur with the fats and oils. They differ from most of the alcohols previously discussed in being not only insoluble in water, but soluble in fat-solvents such as ether and chloroform. They contain a complex cyclic structure, and are discussed on p. 54. **Aromatic alcohols** occur in small amounts in the essential oils of some plants (Chap. XVIII), and other **cyclic alcohols** occur among the *terpenes* (Chap. XXI).

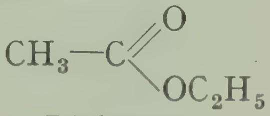
CHAPTER IV

FATTY ACIDS

Structure and General Properties. The simplest aliphatic acids are **monobasic** acids which form a homologous series of general formula $C_nH_{2n+1}\cdot COOH$, with formic acid, $H\cdot COOH$, as the first member ($n = 0$). They may be regarded as oxidation products of the corresponding series of monohydric primary alcohols (p. 19). The acidity is due to the **carboxyl** group (—COOH), which forms salts with both inorganic and organic bases, and forms esters with alcohols; in all these cases the hydrogen of the hydroxyl (—OH) group is replaced, as shown in the accompanying formulæ:


Acetic acid


Sodium acetate


Ethyl acetate

The water-soluble acids turn blue litmus red, and are stronger than carbonic acid, liberating carbon dioxide from a solution of sodium carbonate. The first members of the series—formic, acetic, and propionic acids—are liquids, miscible with water in all proportions. Butyric acid and the immediately succeeding members of the series are oily liquids with a disagreeable odour resembling rancid butter. Up to capric acid (C_{10}) they are volatile in steam, and can therefore be separated from the still higher members of the series, which are colourless, waxy, low-melting solids, non-volatile in steam. The acidity of the series decreases with increasing molecular weight.

Occurrence. The first members of the series are not present in plants to any large extent. Some of them occur as *esters* in **essential oils**; also, since these acids are closely related to amino-acids, the fundamental units of the protein molecule, they may also play an important part in the metabolism of nitrogenous compounds. The higher members of the series (Table I) are present in both plant and animal **fats** and **oils** as *esters* of *glycerol*, and hence the whole series acquired the name of the 'fatty' acids. Still more complex members of the series occur as *esters* of various alcohols in the **waxes.**

Formic Acid

Formic acid, $H\cdot COOH$, is secreted by the hairs of the stinging Nettle (*Urtica dioica*), and also by ants (*Formica*). It is a colourless liquid with a pungent odour. Unlike any other fatty acid, it and its salts contain an aldehydic group ($H\cdot\dot{C}:O$), and can therefore be distinguished by their reducing properties, *e.g.* with ammoniacal silver nitrate (p. 57). The ester, **amyl formate,** occurs in the fruit of the Apple (*Pyrus Malus*).

Acetic Acid

Acetic acid, $CH_3\cdot COOH$, occurs in *pyroligneous acid* from the destructive distillation of wood (p. 21). A dilute solution of acetic acid, known as **vinegar,** is prepared commercially by the bacterial oxidation of ethyl alcohol, and this oxidation also occurs naturally in the souring of wine and other alcoholic liquors on exposure to air. The organism responsible is *Mycoderma aceti*. Acetic acid is a colourless liquid; in the pure anhydrous form (*glacial acetic acid*) it forms colourless crystals, m.p. 16·7° C. **Amyl acetate,** a fragrant liquid, occurs in the essential oils of several fruits, including the Apple and the Banana (*Musa sapientum*).

EXPT. 8. *Acetic Acid*

1. Add sodium carbonate solution to a little acetic acid in a test-tube, and note the evolution of carbon dioxide with effervescence.

2. Prepare a neutral solution by adding ammonium hydroxide to acetic acid until the solution is just alkaline, and boil off the excess ammonia. On the addition of ferric chloride solution a red liquid is formed, and, on boiling, a red-brown precipitate of basic ferric acetate is thrown down.

3. Formation of ethyl acetate (p. 24).

Acetates

1. Warm the solid or a concentrated aqueous solution with dilute sulphuric acid. Acetic acid is evolved, recognised by its odour.

2. Add ferric chloride solution to a solution of the salt, and boil; ferric acetate is precipitated.

3. Repeat test (3) above, using the solid or an aqueous solution.

Hydrolysis of Ethyl Acetate

Place 10 grm. of ethyl acetate in a round-bottomed flask fitted with a condenser set for reflux, and add 30 c.c. of 20 per cent. aqueous sodium hydroxide. Boil gently over wire gauze until no odour of ethyl acetate is observed. Set the condenser for distillation, and distil over about 10 c.c. Test the distillate for ethyl alcohol by the iodoform reaction (p. 24). Pour the residue from the flask into an evaporating dish, neutralise the excess alkali with dilute hydrochloric acid, and evaporate to dryness on a water-bath. Test the residue for an acetate.

Other Members of the Series, $C_nH_{2n+1}\cdot COOH$

Propionic acid, $C_2H_5\cdot COOH$, is present in pyroligenous acid. Two isometric butyric acids are possible, *n*-**butyric acid,** $CH_3\cdot(CH_2)_2\cdot$

COOH, which occurs in some animal fats as a glyceride, and *iso*-**butyric acid,** $(CH_3)_2\cdot CH\cdot COOH$, which occurs in plants, especially fruits, as fragrant esters. Of the valeric acids, the most common in plants is *iso*-**valeric acid,** $(CH_3)_2\cdot CH\cdot CH_2\cdot COOH$, which occurs particularly in species of the Valerian (*Valeriana*).

All the other important members of this series occur combined as *glyceryl esters* in the **fats** and **oils,** and some also occur either *free* or as *esters* in the **waxes;** they can be obtained by *hydrolysis* of these esters. Table I contains a list of these fatty acids, with information concerning the chief plant sources of their esters. It will be seen that the molecules of these acids all contain an *even* number of carbon atoms. Two important members are **palmitic acid** and **stearic acid,** which are both crystalline solids. Palmitic acid was first obtained by the hydrolysis of palm oil (p. 41), but the main source is now Japan 'wax,' which is not a true wax but a greenish fat obtained in from 15 to 25 per cent. yield from berries of various species of Sumach (*Rhus*). Stearic acid can be made from mutton fat (tallow); another important source is Shea 'butter,' a fat occurring to the extent of 45 to 55 per cent. in the

TABLE I

Fatty acids.	Formula.	Per cent. of acids from plant fats.
C_6 Caproic	$CH_3\cdot(CH_2)_4\cdot COOH$	Coconut and palm kernel oils (2 per cent.).
C_8 Caprylic	$CH_3\cdot(CH_2)_6\cdot COOH$	Coconut (8 per cent.) and palm kernel (3 per cent.) oils.
C_{10} Capric	$CH_3\cdot(CH_2)_8\cdot COOH$	Coconut (8 per cent.) and palm kernel (6 per cent.) oils.
C_{12} Lauric	$CH_3\cdot(CH_2)_{10}\cdot COOH$	Coconut (48 per cent.), palm kernel (50 per cent.), and laurel (35 per cent.) oils.
C_{14} Myristic	$CH_3\cdot(CH_2)_{12}\cdot COOH$	Nutmeg oil (73 per cent.), myrtle 'wax' (58 per cent.), coconut and palm kernel (16 per cent.) oils.
C_{16} Palmitic	$CH_3\cdot(CH_2)_{14}\cdot COOH$	Palm oil (30–40 per cent.), Japan 'wax,' and myrtle 'wax' (36 per cent.).
C_{18} Stearic	$CH_3\cdot(CH_2)_{16}\cdot COOH$	Cacao butter (33 per cent.), Shea butter (35 per cent.).
C_{20} Arachidic	$CH_3\cdot(CH_2)_{18}\cdot COOH$	Macasser [1] oil (19 per cent.), peanut oil (5 per cent.).
C_{22} Behenic	$CH_3\cdot(CH_2)_{20}\cdot COOH$	Ben [2] oil.
C_{24} Lignoceric	$CH_3\cdot(CH_2)_{22}\cdot COOH$	Peanut oil (3 per cent.); carnaüba wax, Pisang wax.
C_{26} Cerotic Higher homologues	$CH_3\cdot(CH_2)_{24}\cdot COOH$	Poppy wax, carnaüba wax.

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[1] Macasser oil is obtained from a tree, *Schleichera trijuga*, of the *Sapindaceæ*.

[2] Ben oil is expressed from the seeds of species of *Morigna* of the *Leguminosæ*.

nuts of the tree *Butyrospermum Parkii*, a native of the Sudan. The free acids are used in the manufacture of **candles** (p. 39), while their **sodium** and **potassium salts,** prepared by hydrolysis of the natural fat with the appropriate alkali, are used as **soaps.** Sodium salts give a *hard* soap, potassium salts a *soft* soap, and both form colloidal solutions in water, giving a stable lather. The calcium and magnesium salts of the fatty acids are insoluble in water, and hence form a curd when soap is used with hard water. Stearates of calcium, magnesium, aluminium, and zinc are used commercially in waterproofing textiles and in 'flattening' wall-paints.

EXPT. 9. *Stearic Acid*

1. To a little solid stearic acid add sodium carbonate solution and warm gently. Note the effervescence of carbon dioxide, and that the resulting solution of the sodium salt lathers on shaking.
2. Show that an ethereal solution of the acid does not decolorise bromine water.

Stearates

1. Show that a freshly made solution of pure soap in water has an alkaline reaction to phenolphthalein due to the partial hydrolysis of the salt.
2. To separate portions of a soap solution add
   - (*a*) dilute HCl—the fatty acids are precipitated;
   - (*b*) $CaCl_2$ solution—the insoluble calcium salt separates;
   - (*c*) $MgSO_4$ solution—the insoluble magnesium salt separates;
   - (*d*) solid NaCl—the soap is 'salted out' as a curd.

OTHER ACIDS IN PLANT FATS AND OILS

In addition to the saturated fatty acids discussed above, both plant and animal fats and oils contain **unsaturated monobasic acids** combined with glycerol. An unsaturated compound is one in which the molecule contains less hydrogen than is required to saturate the valencies of the carbon atoms; both *double bonds* (C:C) and *triple bonds* (C:C) are possible. The unsaturated acids under discussion contain one or more double bonds in the molecule, and are usually grouped into corresponding series:

I. Oleic series: one double bond: $C_nH_{2n-1}\cdot COOH$.
II. Linoleic series: two double bonds: $C_nH_{2n-3}\cdot COOH$.
III. Linolenic series: three double bonds: $C_nH_{2n-5}\cdot COOH$.

They are all oily liquids, insoluble in water.

A few **hydroxy-acids**—that is, acids in which one or more hydrogen atoms are replaced by hydroxyl groups—also occur as glycerides, and **dibasic acids** of the series $HOOH\cdot(CH_2)_n\cdot COOH$ occur in Japan 'wax.' All these acids are 'straight-chain' or *normal acids*. Finally, a group of related **cyclic acids** containing a five-membered ring structure occur in oils from tropical plants belonging to the natural order *Flacourtiaceæ*.

Individual acids of these types will now be discussed briefly. It is a significant fact that the most important and most widely distributed acids of all these types contain **eighteen** carbon atoms in the molecule. Like the saturated series, these acids are obtained by *hydrolysis* of their glyceryl esters, the fats and oils.

Unsaturated Monobasic Acids

The Oleic Series, $C_nH_{2n-1}\cdot COOH$. The most important member of the series is **oleic acid,** $C_{17}H_{33}\cdot COOH$. This formula has two hydrogen atoms less than stearic acid, and therefore contains one double bond. Several isomers are possible, depending on the position of this bond. At least two such isomers occur naturally, *viz.* oleic acid itself, with the double bond between carbon atoms 9 and 10 (numbering from the carboxyl carbon), $CH_3\cdot(CH_2)_7\cdot CH{:}CH\cdot(CH_2)_7\cdot COOH$, which occurs in practically all fats and oils, but predominates in olive oil (70–85 per cent. of the acids), and **petroselinic acid,** with the double bond between carbon atoms 6 and 7, $CH_3\cdot(CH_2)_{10}\cdot CH{:}CH\cdot(CH_2)_4\cdot COOH$, which occurs in the seed oils from several species of *Umbelliferæ* (76 per cent. in Parsley seed oil). Oleic acid is a colourless oil when pure, but is obtained as a reddish-brown liquid on being separated by pressing from the mixture of acids obtained on hydrolysis of a natural oil. Oleic acid is insoluble in water, but, like the saturated fatty acids, it gives rise to soaps with alkalis. The presence of a double bond confers on a molecule greater chemical reactivity, and especially the capacity to form **additive** compounds. Oleic acid absorbs one molecular proportion of *bromine* at the double bond to form a dibromo-derivative, $—CHBr\cdot CHBr—$, and also absorbs *oxygen*, giving a resinous material. In the presence of a catalyst (reduced nickel is used in technical practice), oleic acid combines with one molecular proportion of *hydrogen*, and is thus converted into solid stearic acid. This process is called **catalytic hydrogenation.**

Expt. 10. *Oleic Acid*

1. Show that oleic acid dissolves in sodium carbonate solution with effervescence, and that the resultant solution lathers on shaking.

2. Show that an ethereal solution of oleic acid decolorises bromine water.

Another member of this series is **erucic acid,** a C_{22}-acid which is characteristic of the oils of several species of *Cruciferæ,* including mustard seed and rape oils. It contains a double bond between carbon atoms 13 and 14, the formula being $CH_3{\cdot}(CH_2)_7{\cdot}CH{\vdots}CH{\cdot}(CH_2)_{11}{\cdot}COOH$. A simple member of this series, **tiglic acid,** with an uneven number of carbon atoms and a branched chain, $CH_3{\cdot}CH{\vdots}C(CH_3){\cdot}COOH$, occurs in croton oil, but in the free state, and not as a glyceride.

The Linoleic Series, $C_nH_{2n-3}{\cdot}COOH$. This series contains two double bonds per molecule, and all such acids occurring in the fats and oils are isomeric C_{18}-acids, the isomerism depending on the position of the double bonds. **Linoleic acid,** $C_{17}H_{31}{\cdot}COOH$, with the double bonds in the 9, 10 and 12, 13 positions, is the most widely distributed acid in plant fats and oils. It occurs especially in linseed (48 per cent. of the total acids) and cotton-seed (40 per cent.) oils. A simple member of this series, **sorbic acid,** $CH_3{\cdot}CH{\vdots}CH{\cdot}CH{\vdots}CH{\cdot}COOH$, occurs free in the berries of the Mountain Ash (*Pyrus Aucuparia*).

The Linolenic Series, $C_nH_{2n-5}{\cdot}COOH$. This group contains three double bonds in the molecule, and, like the linoleic series, consists of isomeric C_{18}-acids. **Linolenic acid,** $C_{17}H_{29}{\cdot}COOH$, occurs in many plant oils, and an isomer, **elæostearic acid,** occurs in tung oil, and has the structure $CH_3(CH_2)_3{\cdot}CH{\vdots}CH{\cdot}CH{\vdots}CH{\cdot}CH{\vdots}CH{\cdot}(CH_2)_7{\cdot}COOH$.

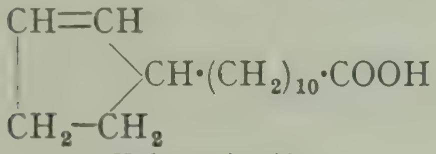
Tariric acid, $CH_3{\cdot}(CH_2)_7{\cdot}C{\vdots}C{\cdot}(CH_2)_7{\cdot}COOH$, is the only naturally occurring fatty acid containing a triple bond. It is found only in the seeds of *Picramnia.*

Monobasic Hydroxy-Acids

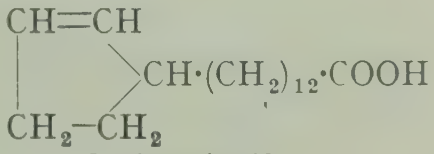
Two saturated hydroxy-acids occur in plant oils. **Sabinic acid** is a monohydroxypalmitic acid, $C_{15}H_{30}(OH){\cdot}COOH$, and occurs in the seed oils of some of the *Coniferæ.* **Dihydroxystearic acid,** $C_{17}H_{33}(OH)_2{\cdot}COOH$, occurs to the extent of 1 per cent. in castor oil. The most important hydroxy-acid occurring as a glyceride in plants is **ricinoleic acid,** $C_{17}H_{32}(OH){\cdot}COOH$, an *unsaturated* monohydroxy-acid related to oleic acid. It forms over 80 per cent. of the total acids in castor oil.

Cyclic Acids

Three cyclic acids occur in the oils from various tropical species of the *Flacourtiaceæ,* the most important of which is chaulmoogra oil. The acids have been named **hydnocarpic,** $C_{16}H_{28}O_2$, **chaulmoogric,** $C_{18}H_{32}O_2$, and **gorlic,** $C_{18}H_{30}O_2$, acids. The formulæ of the first two have been established by synthesis (Adams), and are given below. Gorlic acid contains the same ring structure, with an unsaturated side-chain, —$(C_{12}H_{22})$·COOH.



Hydnocarpic acid



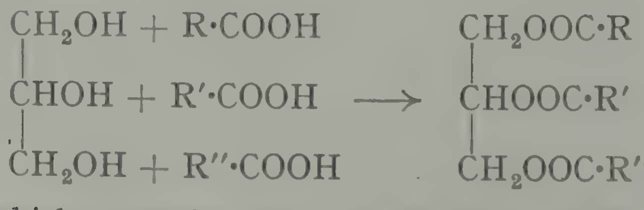
Chaulmoogric acid

CHAPTER V

FATS AND OILS

Distribution. The fats and oils comprise one of the most important groups of storage compounds or **reserve food material** in plants (and the only one in animals). In plants they are found mainly in seeds, fruits, and spores, but they also occur to a less extent in leaves, roots, tubers, and other vegetative organs. Storage fat usually occurs in globules, which may form more than 50 per cent. of the dry weight of the tissue; but fat or oil may also occur in an emulsified form in the protoplasm and in the latex of some plants. The high percentage of oil-containing seeds in nature is often incompletely recognised, because of the cultivation in temperate climates of starch-storing seeds (grain) for human consumption. If the flora of the tropics are taken into consideration, oil-storing seeds predominate to the extent of about 90 per cent. of all plants. The percentage of fat or oil, however, varies in different natural orders, as will be seen in the more detailed account of the important plant oils (p. 41), and these oils may also be associated with other reserve food materials. For example, in the *Palmæ*, the Coconut Palm (*Cocos nucifera*) contains about 67 per cent. of oil in the kernel, and associated with it are hemicelluloses, while the Oil Palm (*Elæis guineensis*) contains up to 70 per cent. of oil in the fleshy portion of the fruit (pericarp) and about 50 per cent. of a fat in the kernel. Oil is stored in seeds of the *Coniferæ* together with sugars, and in the *Gramineæ* it occurs with starch, the oil being stored mainly in the embryo.

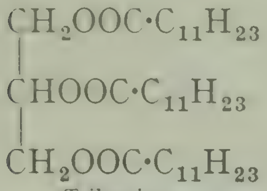
Chemical Structure. Chemically, the fats and oils are **mixtures** of **esters** of the same alcohol, **glycerol,** with the various acids discussed in the preceding chapter. It will be seen from the following formula that glycerol, being a trihydric alcohol, can form esters with each alcoholic group, *i.e.* it may react with one, two, or three molecular proportions of acid. In the fats and oils only tri-esters (triglycerides) occur.



$$\begin{array}{lcl} CH_2OH + R\cdot COOH & & CH_2OOC\cdot R \\ | & & | \\ CHOH + R'\cdot COOH & \longrightarrow & CHOOC\cdot R' \\ | & & | \\ CH_2OH + R''\cdot COOH & & CH_2OOC\cdot R'' \end{array}$$

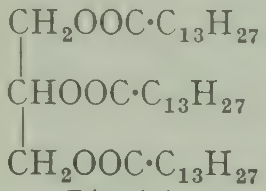
Glycerides which contain three molecules of the same acid

($R = R' = R''$) are called *simple glycerides*, examples in plant fats and oils being trilaurin, trimyristin, and triolein :



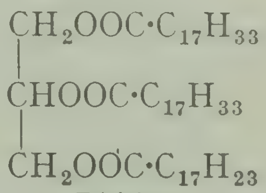
$$\begin{array}{l} CH_2OOC\cdot C_{11}H_{23} \\ | \\ CHOOC\cdot C_{11}H_{23} \\ | \\ CH_2OOC\cdot C_{11}H_{23} \end{array}$$

Trilaurin, or
Glyceryl trilaurate



$$\begin{array}{l} CH_2OOC\cdot C_{13}H_{27} \\ | \\ CHOOC\cdot C_{13}H_{27} \\ | \\ CH_2OOC\cdot C_{13}H_{27} \end{array}$$

Trimyristin, or
Glyceryl trimyristate



$$\begin{array}{l} CH_2OOC\cdot C_{17}H_{33} \\ | \\ CHOOC\cdot C_{17}H_{33} \\ | \\ CH_2OOC\cdot C_{17}H_{23} \end{array}$$

Triolein, or
Glyceryl trioleate

Mixed glycerides are also possible, represented by the general formula above, where R, R′, and R″ are different radicals. Some of these glycerides contain only saturated acids, some only unsaturated, while others contain both saturated and unsaturated acids in the same molecule. Glycerides of the unsaturated acids such as linoleic acid (a liquid) are also liquid, while glycerides of the saturated acids (mostly solid) tend to be solid also. There are discrepancies in the nomenclature of some natural products due to their being named before their chemical constitution was understood, *e.g.* Japan 'wax' and myrtle 'wax' are both mixtures of glycerides and therefore fats, while sperm 'oil' from the sperm whale is not an oil but a wax. It must also be remembered that the oils here discussed are the 'fixed' or non-volatile oils, and are distinct chemically and in physiological function from the volatile or 'essential' oils.

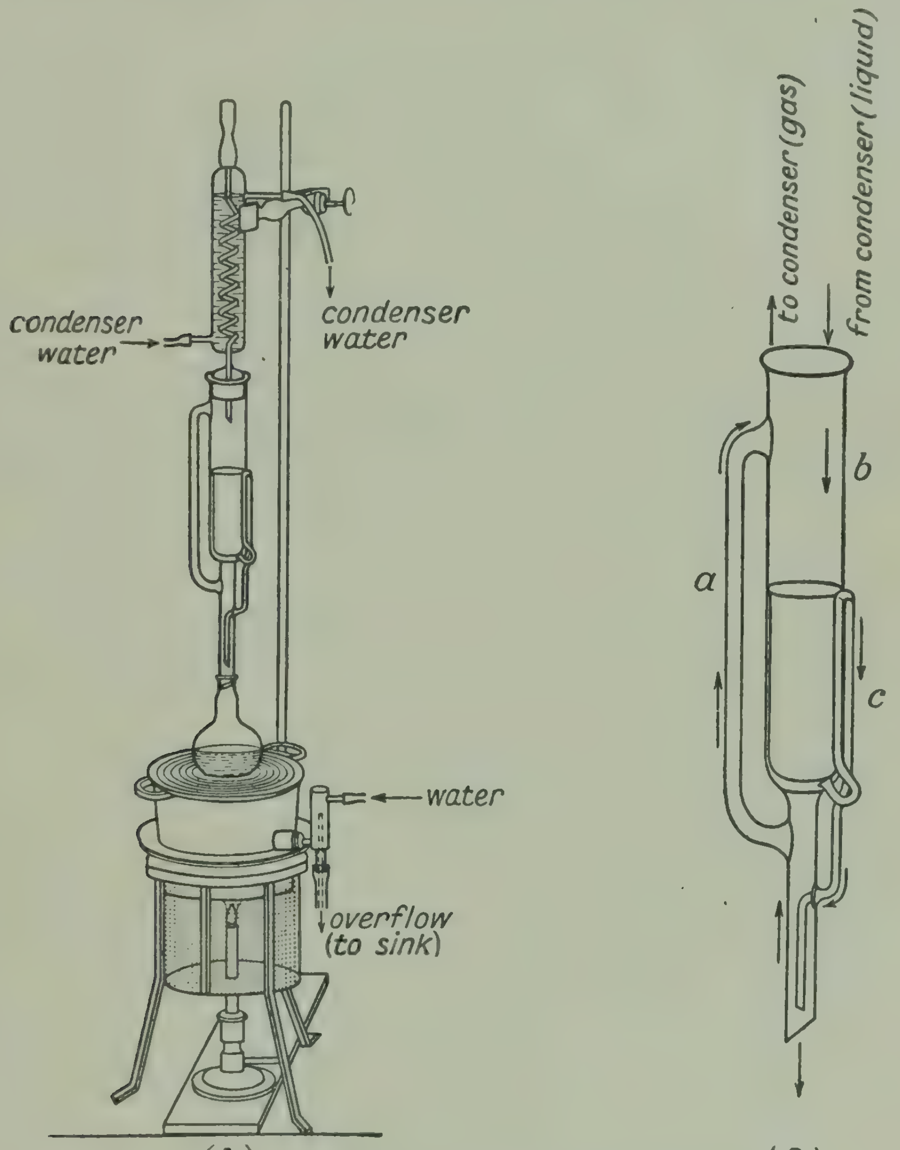
The distinction between fats and oils is to some extent a physical one; a fat is solid or semi-solid at ordinary temperatures, but may be an oil in the tropics. The chemical difference between fats and oils depends partly on the *amount of unsaturation*, an oil containing a higher proportion of unsaturated acids, especially those with two or three double bonds, than a fat. The *percentage of fatty acids of high molecular weight* is another differentiating factor: fats contain more palmitic and stearic acid than oils, in which lauric acid may predominate. Animals, in contrast to plants, tend to build up solid fats. **Climate** influences the type of fat or oil stored by plants. Fats from *tropical* plants contain a relatively high proportion of *saturated acids*, as compared with those from colder climates; thus, coconut oil contains 92 per cent. of saturated acids as glycerides, while linseed oil contains only about 10 per cent.

The fats of **growing tissues** (leaf and stem), of roots, and of the fruit coats of all plants are very similar in chemical composition, as they all contain **palmitic, oleic,** and **linoleic** acids as their major fatty acid components. Leaf fats, particularly of grasses, contain the highest proportion of unsaturation (Chibnall).

Seed fats can be divided into two types. The first group are composed of these same glycerides. Hilditch has subdivided this group according to the proportions of fatty acids present, and has correlated this with the botanical families in which the acids predominate. For instance, the *Coniferæ* are representatives of a group in which linoleic acid predominates, while the *Gramineæ* and *Malvaceæ* contain palmitic acid as a major component. On the other hand, there is a second type of seed fats in plants belonging to the same or closely allied **natural orders** which contain the **same distinctive fatty acids.** Several of the natural orders are characterised chemically, in that one or more fatty acids predominate in the oils of all the species. The most important examples are the predominance of **lauric acid** in the kernel fats of the *Palmæ*; **myristic acid** in the kernel fats of the *Myristicaceæ*; **erucic acid** in the seed fats of the *Cruciferæ*; **petroselinic acid** in the seed fats of the *Umbelliferæ* and in the closely related plant, the common Ivy (*Hedera Helix*) of the *Araliaceæ*, and the **cyclic acids** (chaulmoogric group) in the *Flacourtiaceæ*. This is not universal, however; in the *Euphorbiaceæ*, for example, castor oil from seeds of *Ricinus communis* is entirely different from the oils of the genera *Aleurites* and *Mercurialis*. The predominance of one acid in the oils from a natural order is confined only to the seed or kernel oils, and the oil or fat from other parts of the plant may be entirely different, *e.g.* palm oil from the fruit pulp of the Oil Palm contains no lauric acid.

Hilditch has devised a useful method for separating the glycerides in the oils, and therefore for determining the exact manner in which the fatty acids are grouped together in the glycerides. This work has shown that in the seed fats there is often a similarity even between the actual glycerides present in closely related plants, and that in almost all fats there tends to be an *even distribution* of the various fatty acids throughout the glycerides. There are a few exceptions to this *mixed glyceride rule.* In the plant fats these are two fruit-coat fats, palm oil and olive oil, and two seed fats, *viz.* laurel oil from *Laurus nobilis*, and the oil from *Myristica malabarica.* In the animal world the exceptions are the depot and milk fats of the *Ungulata* (sheep, pig, etc.).

Isolation. Fats and oils are all insoluble in water, sparingly soluble in alcohol, and readily soluble in the 'fat-solvents,' *viz.* ether, chloroform, carbon disulphide, and benzene. There are three types of extractive methods: (*a*) The oldest and simplest method is by melting the fat from the tissue by using boiling water, so that the cell-walls are ruptured and the oil rises to the surface, whence it may be skimmed off. This method is mostly used for animal fats, but it is also used in the preparation of myrtle 'wax,' and sometimes of castor and olive oils. (*b*) The common method with plant products is the use of pressure (usually hydraulic). Nuts and large seeds are first *decorticated*; that is, the shells are removed from their kernels, and the latter are then crushed or rolled (*milling*). If the ground seeds are pressed at ordinary temperatures, *cold-drawn* oil, mostly used for food, is obtained; the *press-cake* is then heated at steam-heat and passed into the presses again, giving *hot-drawn* oil, mainly used for soap-making. Sometimes only one hot pressing is made. The residue from the presses contains protein and some residual oil, and is used for stock-feed, *e.g.* linseed



(A) (B)

FIG. 3. Soxhlet's Extraction Apparatus

cake and meal, cottonseed cake. (*c*) The third method consists in extracting the oil with a suitable solvent such as benzene, gasoline, carbon disulphide, or di- or tri-chlorethylene in a continuous extraction process, followed by the removal and recovery of the solvent by distillation. This method results in the most complete extraction of the oil, but if the residue is to be used as a stock-feed it must be carefully freed from all traces of solvent. The same method is used in the laboratory for the estimation of fats in tissues, the solvent being ether, and a continuous extraction apparatus known as a *Soxhlet Extractor* is used.

EXPT. 11. *Determination of the Percentage of Fat in Nuts*

Weigh out 10 grm. of ground almonds, hazel, or other nuts, and place in the thimble of a Soxhlet apparatus (fig. 3). Place 200 c.c. of ether in a 500-c.c. round-bottomed or conical flask (previously weighed), and reflux on the water-bath for one hour. Disconnect the flask, set the condenser with the flask for distillation, and distil off the ether from a water-bath, with the flame removed. The last traces of ether and any water present are removed by placing the flask in a desiccator, and evacuating. The flask and its contents are finally weighed. Calculate the percentage of fat in the material used.

Properties and Identification. Since the fats and oils consist of mixtures of glycerides, they have no exact melting-point. Some of the plant products are solid or semi-solid fats, *e.g.* myrtle 'wax,' m.p. 40–46° C., and cacao 'butter,' m.p. 30–34° C.; others, such as castor and linseed oils, are liquid. Some oils from the leaves of conifers, especially those growing in northerly regions, remain liquid at very low temperatures. Most of the fats and oils are optically inactive unless they contain an optically active acid, *e.g.* the cyclic acids. When pure, the fats and oils are odourless and tasteless, but the natural products have usually a distinctive flavour and often colour, due to traces of decomposition products and other substances, especially the essential oils. They give a few characteristic colour reactions, *viz.* a red colour with the pigment from *Alkanet* root, and pink colours with the stains *Sudan III* and *Scharlach R.* The main criterion for the identification of any particular fat is the correspondence of various numbers or values, *e.g.* the saponification value and the iodine number (*vide infra*), determined by chemical treatment of the material in question, followed by reference to tables of standard values.

EXPT. 12. *Fats and Oils*

(Use linseed oil, olive oil, and cacao butter.)

1. Show that they are insoluble in water and alcohol, but soluble in ether and chloroform.

2. Add a piece of alkanet root to a fat or oil and warm on the water-bath; a red colour is developed.

Chemically the fats and oils are neutral. Two important reactions are (i) hydrolysis, since they are esters, and (ii) the formation of addition products, due to the presence of unsaturated acids as glycerides.

Hydrolysis. The hydrolysis of the fats and oils is the basis of two commercial processes, the manufacture of candles from the free fatty acids, and the manufacture of soaps. In both cases glycerol is an important by-product. There are three methods of hydrolysing fats to obtain the free fatty acids: (*a*) Hydrolysis with superheated steam under pressure in the presence of a metallic oxide as catalyst (lime, magnesium oxide, or zinc oxide); (*b*) hydrolysis by warming with dilute sulphuric acid usually in the presence of naphthalene sulphonic acid as a catalyst (Twitchell's process); (*c*) the latest method is the use of **lipase,** an enzyme occurring in many oily seeds and obtainable in quantity from castor seed; this hydrolyses fats to glycerol and fatty acids at ordinary temperatures (p. 48). The mixed fatty acids obtained by hydrolysis are separated by pressure into liquid and solid portions; the latter contains mainly palmitic and stearic acids and is used in *candle-making*. The liquid acids, which are mostly unsaturated, may then be hydrogenated to solid saturated acids (p. 31). In *soap-making*, the fat or oil is hydrolysed by boiling with an alkaline reagent (**saponification**). If sodium hydroxide is used, the sodium salts, *hard soaps*, are salted out by the addition of common salt, which throws the soap out of solution; with potassium hydroxide, the potassium salts on cooling separate as a jelly containing some glycerol, forming a *soft soap*.

EXPT. 13. *Saponification of Linseed (or Olive) Oil*

Place 5 c.c. of oil in a boiling-tube with 20 c.c. of 10 per cent. alcoholic caustic soda (prepared by dissolving sodium hydroxide in the minimum amount of water and making up the volume with alcohol), shake well, then warm in a beaker of hot water until a drop of the liquid withdrawn on the end of a glass rod gives no milkiness with water. Transfer the liquid to an evaporating basin, and boil off the alcohol on a water-bath. Add a saturated solution of sodium chloride, and filter off the sodium salts of the fatty acids (soap). Test the precipitate as on p. 30 (stearates). Neutralise the filtrate from the salting out with dilute hydrochloric acid, and evaporate to a syrup. Add alcohol to throw out the sodium chloride, filter, and evaporate off the alcohol. Test the residue for glycerol (p. 25).

Hydrolysis with alkali is utilised in the characterisation of fats and oils by the **saponification value,** defined as the number of milligrams of potassium hydroxide required to saponify completely 1 grm. of the fat or oil. Examples are castor oil, S.V. 177–187; linseed oil, S.V. 189–196; myrtle 'wax,' S.V. 205–212; coconut oil, S.V. 251–263.

EXPT. 14. *Determination of the Saponification Value of a Fat*

Approximately 0·5 *N*-alcoholic KOH is prepared by dissolving 14 grm. of KOH in the minimum amount of water, and diluting to 500 c.c. with alcohol; after 24 hours the solution is filtered. 1·5 to 2 grm. of the fat or oil is weighed into a 200-c.c. round-bottomed flask, 25 c.c. of the alcoholic KOH introduced by means of a pipette, then about 20 c.c. of alcohol added, and the mixture heated on a water-bath under reflux for about 30 minutes. A blank experiment with the alcoholic alkali alone is run in parallel. The flasks must be shaken from time to time, and when a clear solution is obtained, one drop of phenolphthalein is added, and each solution is titrated with 0·5 *N*-HCl. The difference between the control and the saponification experiments is the equivalent of the alkali required to saponify the fat. Calculate the saponification value in milligrams of alkali required by 1 grm. of fat.

Addition Products. Just as the unsaturated acids, by virtue of one or more double bonds in the molecule, can absorb oxygen, so the glycerides of such acids absorb oxygen (technically called 'drying'), forming resinous materials. Plant fats and oils differ in this property, depending on the proportion of unsaturated acids which they contain, and are therefore divided into *non-drying*, *semi-drying*, and *drying* oils. The oils will also absorb bromine or iodine at these unsaturated linkages, and hence the amount of unsaturation is measured by the **iodine number,** the amount of iodine in grams absorbed by 100 grm. of oil. In many cases all the oils from one natural order fall into only one of these divisions, and hence this classification has been used here. One exception has already been referred to, *viz.* in the *Euphorbiaceæ*, castor oil is a non-drying oil, I. No. 82–90, while tung oil is a drying oil, I. No. 157–170. The economic uses of the fats and oils depend primarily on this division. Coconut oil, I. No. 8–9·6, cacao butter, 34–40, olive oil, 77–94, and palm oil, 48–58, are non-drying oils (I. No. < 100), and are used mainly for edible purposes; cotton-seed oil, 100–115, and rape (colza) oil, 94–106, are semi-drying oils (I. No. between 100 and 130), and are used partly for edible purposes and partly for soap-making; while linseed oil, 170–204, and tung oil, 147–170, are the characteristic drying oils (I. No. > 130) and are used in the manufacture of paints, varnishes, and linoleums.

EXPT. 15. *Drying and Non-drying Oils*

Dissolve linseed and olive oils in a little ether in two separatory funnels and shake with bromine water; note the rapid absorption of bromine, and the separation of a solid bromo-derivative, especially with linseed oil.

Again, just as the unsaturated acids can be catalytically hydrogenated to give saturated acids (p. 31), so unsaturated oils can be *hardened* to give semi-solid or solid fats, depending on the degree of hydrogenation. In this way, semi-drying oils such as cotton-seed oil can be converted to fats with melting-points about 60° C., which are used as substitutes for butter and lard.

Some Important Plant Fats and Oils

1. Non-drying Oils

Palmæ. The palms yield two different types of oils: the *pulp oils*, obtained from the fleshy portion of the fruit, and the *kernel* or *nut oils* from the kernels of the fruit. The former are liquid or semi-solid and often highly coloured, and contain no lauric acid; the latter are pale-coloured or white solid fats, characterised by a very high content of saturated fatty acids and the predominance of lauric acid. The Oil Palm (*Elæis guineensis*) gives **palm oil** (a pulp oil) and **palm kernel oil**; the difference in composition can be seen from the following table, which gives the percentage of the various fatty acids present:—

TABLE II

Percentage of Acids from (*a*) Palm Oil, (*b*) Palm Kernel Oil.

	Caprylic.	Capric.	Lauric.	Myristic.	Palmitic.	Stearic.	Oleic.	Linoleic.
(*a*)	—	—	—	—	30–40	4–6	38–50	7·5–10·7
(*b*)	3	6	50	16	6·5	1	16·5	1

Palm oil consists mainly of mono-oleo-dipalmitin and dioleomonopalmitin with about 6 per cent. each of tripalmitin and triolein. It is used for edible purposes, in soap-making, and as a fuel in Diesel motors (Belgian Congo). Palm kernel oil contains mainly dilauro-myristin, and no simple glycerides. **Coconut oil** is a kernel oil from the Coconut Palm (*Cocos nucifera*), and *copra* is the sun-dried kernel of the coconut from which the oil is afterwards expressed. Like palm kernel oil, it consists mainly of dilauromyristin.

Oleaceæ. **Olive oil** is obtained from the fruit of the tree *Olea* *europæa.* The oil content depends on the variety of the species, the type of soil, and the climatic conditions. Oleic acid forms from 70 to 85 per cent. of the total fatty acids.

Myristicaceæ. The Mace family comprises tropical trees, the fruits of which enclose seeds containing up to 30 per cent. of fat. These fats are usually quite hard, and contain large amounts of glycerides of myristic acid. The Nutmeg (*Myristica fragrans*) is commercially the most important; the fat, m.p. 42—52° C., which contains 73 per cent. of myristic acid, is used for medicinal purposes and for flavouring.

Flacourtiaceæ (or *Bixineæ*). Various species of this tropical family, some being bushes, others trees, yield characteristic kernel oils containing the cyclic acids (p. 32) as glycerides. They have been used by the natives in the treatment of leprosy and other skin diseases. The following are the main species: *Taraktogenos kurzii* (Burma), *Hydnocarpus* (Asia), and *Oncoba* (Africa) yield 48–55 per cent. of oil, the first mentioned being the true **chaulmoogra oil;** *Carpotroche*, *Lindackeria*, and *Mayna* (all from S. America) contain 60–63 per cent. of oil. All these oils contain chaulmoogric acid and probably gorlic acid, but hydnocarpic acid occurs only in some of the species.

Euphorbiaceæ. Most species of this family have seeds which yield drying oils. A notable exception is *Ricinus communis*, which grows wild in most tropical and subtropical regions and is also cultivated; its seeds yield from 35 to 55 per cent. of **castor oil.** This oil is distinguished from all other fixed oils by its ready solubility in alcohol and in glacial acetic acid, and by its high content of ricinoleic acid, which forms as much as 80 per cent. of the total fatty acids. It has wide uses as a lubricant, especially for aircraft and in tropical countries, and is also used in the dyeing industry and in medicine.

Myricaceæ. The berries of various species of Bayberry (*Myrica*) growing in North and South America and South Africa are covered with a solid fat called **bayberry tallow** or **myrtle wax** to the extent of 15–20 per cent. of the weight of the berries. It contains mainly myristic (58 per cent.) and palmitic (36 per cent.) acids as glycerides, and is used in the manufacture of candles and soap.

Sterculiaceæ. The seeds or 'beans' of the Cocoa plant (*Theobroma Cacao*) contain from 50–57 per cent. of a pale yellowish fat, m.p. 30–34° C., and when this is extracted by pressure, the ground press-cake is known as *cocoa.* The fat (**cacao butter**) consists mainly of glycerides of oleic, palmitic, and stearic acids.

Sapotaceæ. **Shea nut oil** or butter (see p. 29).

Anacardiaceæ. **Japan wax** (see pp. 29 and 31).

Rosaceæ. **Almond oil** is obtained to the extent of 40–60 per cent. from the kernels of both the bitter and sweet varieties of the Almond (*Prunus Amygdalus*) and is used in various pharmaceutical preparations. The chief acid obtained by hydrolysis is oleic acid (77 per cent.), with linoleic acid (20 per cent.) and palmitic acid (3 per cent. of the total acids). Kernels of the Peach (*P. Persica*), Plum (*P. domestica*), and Cherry (*P. Cerasus*) yield similar oils.

Fagaceæ. **Hazel** (filbert) **nut oil** is obtained from the kernels of *Corylus Avellana* in 50–60 per cent. yield. It is used for edible purposes and in soap-making.

Leguminosæ. The most important commercial oil is **peanut oil** from the seeds of *Arachis hypogæa*, variously known as peanuts, earthnuts, and groundnuts. It is used for edible purposes generally, has a high content of oleic acid (50–71 per cent. of the total acids), and contains two of the rarer acids, arachidic acid (2·6–5 per cent.) and lignoceric acid (3 per cent.).

2. Semi-drying Oils

Malvaceæ. Seeds of various species of the Cotton plant (*Gossypium*) yield from 28–40 per cent. of **cotton-seed oil,** which forms an important by-product in the preparation of cotton. The seeds are 'delinted'—that is, all small cotton fibres are removed—then decorticated, and finally milled and pressed. The oil contains as glycerides about 70 per cent. of unsaturated acids, especially oleic acid (30 per cent.) and linoleic acid (40 per cent.). It is used in soap-making, and is often hydrogenated and used for cooking purposes.

Cruciferæ. The oils from the seeds of this family are distinguished by containing erucic acid. The seeds of *Brassica Rapa*, *B. Napus*, *B. glauca*, and other cultivated varieties of *B. campestris* yield oils so similar physically and chemically that they are usually grouped together as **rape** or **colza oil.** This is used to some extent as a food, and also as a lubricant and fuel. The seeds yield 30–45 per cent. of oil. Of the acids obtained on hydrolysis, erucic acid constitutes 50–57 per cent., oleic acid 20–32 per cent., and linoleic the rest. White and black mustard seeds (*Brassica alba* and *B. nigra*) contain from 25–30 per cent. of oils, which are very similar to each other and to rape oil in the amounts of the various acids present. Wallflower seeds (*Cheiranthus Cheiri*) give an oil which has somewhat different proportions of these acids, *viz.* 40 per cent. of erucic, 41 of linoleic, and 12 of oleic acids.

Umbelliferæ. The oils from seeds of this family are distinguished by consisting almost exclusively of glycerides of C_{18}-acids, one of which is petroselinic acid. The cultivated species, such as Celery, Parsnip, and Chervil, contain a much higher percentage of petroselinic acid (Table III) than the native wild species, *e.g.* Cow Parsnip.

TABLE III

	Percentage oil in seeds.	Percentage petroselinic acid of total acids.
Parsley (*Petroselinum sativum*) . .	20	76
Fennel (*Fœniculum officinale*) . . .	10	60
Coriander (*Coriandrum sativum*) . . .	20	53
Celery (*Apium graveolens*) . . .	17	51
Parsnip (*Peucedanum sativum*) . . .	—	46
Chervil (*Anthriscus cerefolium*) . . .	13	41
Caraway (*Carum carvi*)	15	26
Cow Parsnip (*Heracleum Sphondylium*) .	—	20

3. Drying Oils

Linaceæ. **Linseed oil** is obtained in 32–43 per cent. yield from the seeds of the Flax plant (*Linum usitatissimum*). In most countries distinct varieties are grown for the fibre (flax) and for oil, except in certain districts of the United States and in the U.S.S.R., where one variety is the source of both. The oil contains a high percentage of unsaturated acids as glycerides, mainly linoleic acid (48 per cent.) and linolenic acid (20–34 per cent.). The special properties of linseed oil are attributed to a mixed glyceride containing two molecules of linolenic acid and one of linoleic acid. The characteristic property of the oil is its absorption of about 29 per cent. of its weight of oxygen to form *linoxyn*, an elastic solid; this property determines its use in the manufacture of varnishes and linoleum.

Euphorbiaceæ. The seeds of many common plants belonging to this natural order, including species of *Mercurialis*, contain drying oils (to about 30 per cent. of the dry weight). The commercial products are, however, obtained from trees of the genus *Aleurites*, **tung oil** (**Chinese wood oil**) being obtained in 30–50 per cent. yield from *A. cordata* and *A. Fordii*, while **candlenut oil** is obtained from *A. moluccana*. Tung oil is important in the manufacture of paints and varnishes, and contains about 86 per cent. of unsaturated acids.

Juglandaceæ. Walnut kernels (*Juglans regia*) contain from

60–70 per cent. of oil, which consists mainly of glycerides of unsaturated acids. It has been used for centuries in the preparation of artists' colours because of its drying properties.

Leguminosæ. The Soy or Soya Bean (*Glycine hispida*) originated in China and is now extensively cultivated in the United States. It yields 22 per cent. of **soybean oil,** the chief acid components of which are oleic acid (32 per cent.) and linoleic acid (49 per cent.). Because of the difficulty of obtaining sufficient quantities of the other drying oils during the recent war, soybean oil has replaced them in many industrial processes, *e.g.* paint manufacture.

Metabolism of the Fats and Oils in Plants

The fats and oils are, as we have seen, mainly storage materials, and represent the greatest concentration of energy among the various types of compounds synthesised in quantity by plants. This is because fats contain more carbon and hydrogen and less oxygen than any of the others, and when they undergo oxidation, a large amount of energy is liberated. A fat yielding the same amount of energy (measured in heat units, *viz.* calories) will take up less space than a carbohydrate; it is significant that in small seeds the storage material is usually oil, whereas in large tubers starch or sugars are the common reserve materials. Even where seeds have endosperm storage of starch, *e.g.* in the *Gramineæ*, the embryo always contains a small amount of fat or oil (about 2–4 per cent. of the dry weight of the whole grain), which furnishes energy for the germinating process.

There are two points at which the metabolism of fat in plants can be studied, *viz.* in the germinating seed and in the ripening seed. In both cases a definite correlation between **fat** and **carbohydrate** has been established.

Germination. Two stages can be distinguished in the germination of fat-storing seeds; in the early stage the total fat content shows little change, but later it rapidly diminishes and the chemical composition of the remaining fat is different in that it contains a considerable amount of *free fatty acids* and there is *less unsaturation* —that is, the iodine number is lower. This last change may be due either to the unsaturated acids being used up first—and this view is justified by the fact that fat disappears more rapidly from seeds containing highly unsaturated oils—or because the unsaturated acids are converted first to saturated or to hydroxy-acids. The final result in all cases is an increase in carbohydrate. An example of the two stages in germination is given by the following results (Leathes) on the Sunflower (*Helianthus annuus*): this contained

56 per cent. fat in the resting seed, the seedling still contained 52 per cent. when the cotyledons reached the surface of the soil, but only 13·5 per cent. remained when the cotyledons were fully expanded. These stages, the change in iodine number, and the increase of carbohydrate are also shown by the following values found in the germination of linseed (Ivanov):—

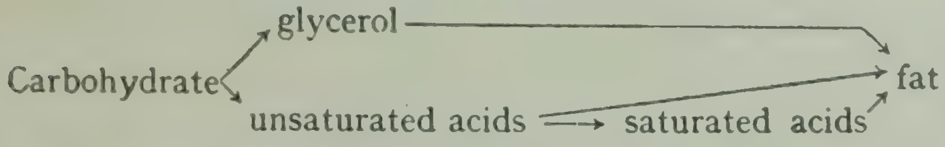
	Fat (per cent.)	Iodine number	Carbohydrate (per cent.)
In seed . . .	33·6,	173·4	4·5
In 4-day seedling . .	26·4	—	6·7
In 8-day seedling . .	16·0	93·4	17·6

Ripening. In the ripening of fat-containing seeds and fruits the fat is formed *in situ*, and as the content of fat or oil increases, that of carbohydrate decreases. This is shown by the change in the respiratory quotient (p. 278), and also by analysis at various stages during ripening, as in the following data for almonds (Leathes):

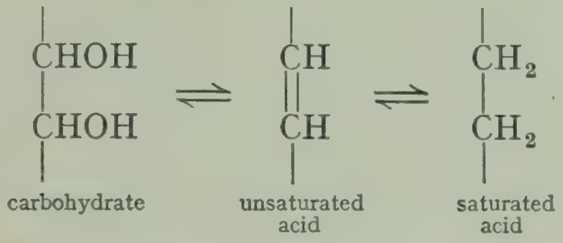
Date.	Oil (per cent.).	Sucrose (per cent.).	Glucose (per cent.).	Starch (per cent.).
June 9 . .	2	6·7	6·0	21·6
July 4 . .	10	4·9	4·2	14·1
Aug. 1 . .	37	2·8	0·0	6·2
Sept. 1 . .	44	2·6	0·0	5·4
Oct. 4 . .	46	2·5	0·0	5·3

Here the oil is produced mainly from starch; whereas in *Ricinus* the sugars, especially glucose, are the source of the fat which develops on ripening. In the early stages of fat synthesis relatively large amounts of the free fatty acids are also present, and it would therefore appear that these acids and glycerol are first formed separately and then combined to give the various mixed glycerides. Glycerol is a product of carbohydrate metabolism in one type of the fermentation process (p. 258), and a similar mechanism may account for its production here, although it never accumulates in the plant. The intervening stages in the development of the fatty acids from the carbohydrates have not yet been established, though various mechanisms have been postulated. It is significant that all the fatty acids in the fats contain an even number of carbon atoms, and that the C_{18} acids predominate, the carbon chain being a multiple of the carbohydrate unit C_6. Glucose breaks down in respiration into simple compounds of two carbon atoms such as acetaldehyde (pp. 60 and 281), and acetaldehyde, or acetyl phosphate derived from it, may condense to form the long 'straight-chain' acids (which would account for the even number), first the unsaturated acids, and then the saturated acids by reduction.

This relationship between fat and carbohydrate may be summarised as follows:—



It will be seen from the following types of grouping that the change from carbohydrate to fat (ripening) is essentially a reduction process, while the reverse reaction (oxidation) occurs in germination:—



$$-CHOH-CHOH- \rightleftharpoons -CH=CH- \rightleftharpoons -CH_2-CH_2-$$

It has already been noted (p. 35) that the fats of tropical plants contain a high percentage of saturated acids compared with those of temperate climates; another statement to the same effect is that all fats found in nature are liquid at the temperatures at which they normally occur, *i.e.* the fats in plants from cold climates must remain liquid throughout the range of temperature to which they are exposed, and therefore must contain a high percentage of unsaturation. This is a *biological reason* for the difference, but some investigators consider that as chemical changes are dependent on temperature, the controlling factor in this case is the temperature reached by the plant, and that in colder climates it is not sufficiently high to effect reduction of the unsaturated acids to the saturated. Ivanov, in Russia, conducted an interesting investigation on the effect of climate upon the composition of fats. He showed that tropical climates favour the formation of oleic acid, while in northern countries or at greater altitudes linolenic acid is developed. Also, plants which contain fats composed of glycerides of highly unsaturated acids are relatively more sensitive to variations of climate than plants the fat of which only contains mono-unsaturated acids, and this is exemplified by the variability in the iodine number of the former type; *e.g.* the farther north that Flax is grown, the higher is the iodine number of the linseed oil.

The conversion of carbohydrate into fat is also seen in many trees, *e.g.* Birches, Plums (especially in the buds), Walnut (in the bark), and Pines (in the needles), where starch disappears and fat accumulates. This change plays an important part in the defence mechanism of the plant against *low temperatures* (p. 318). The presence of fats in leaves, buds, and other superficial parts of the plant may play a similar part to the waxes (p. 49) in protecting the underlying cells from too rapid variations in water-content.

The *cuticle* forming the outermost layer of the epidermis of higher plants is derived from unsaturated fatty acids and their soaps, which have undergone oxidation and condensation at the surface of the tissue (p. 100). The formation of *suberised* tissue admits of a similar explanation.

An *enzyme*, **lipase,** which hydrolyses fats and oils to glycerol and a mixture of acids is widely distributed in plants, especially in fat-storing seeds. The enzyme is comparatively non-specific, hydrolysing other esters in addition to fats. Moreover, various preparations of the enzyme from different plants hydrolyse the fats at different rates (p. 252). The lipase most investigated is obtained from seeds of the Castor plant (*Ricinus*), and is used commercially in the hydrolysis of fats (p. 39). Lipase occurs in the resting seed as a proenzyme (p. 253), which is only active in weak acid solution, a condition which does not exist in the seed. On germination, the true enzyme is developed; this is active in neutral and slightly alkaline media, and the hydrolysis of the stored fat is followed by the changes previously described. There is little doubt that lipase also catalyses the *synthesis* of fats in the ripening process; it has been shown to effect the formation of esters such as ethyl butyrate from ethyl alcohol and butyric acid *in vitro*. In the action of a hydrolysing enzyme such as lipase, the water relationships will be important, and it is significant that **desiccation** is the concomitant of the ripening process, when **synthesis** is taking place, whereas the **absorption of water** characterises germination, when **hydrolysis** occurs.

CHAPTER VI

WAXES, LIPINS, AND STEROLS

In addition to the fats and oils, plants contain relatively small amounts of other substances which are soluble in the fat solvents. Most of them are chemically related to the fats. These are sometimes grouped under the general term **lipides.** We will discuss in turn the **waxes,** the compound lipides (or **lipins**), and the **sterols.**

Waxes

Waxes occur in many plants, generally as a thin deposit on leaves, stems, and fruit. Their main natural function appears to be that of forming a **protective layer,** either against the penetration of water, *e.g.* on leaves of evergreens and the skin of fruits such as plum and grape, or against the loss of water due to excessive evaporation in hot, dry climates. Waxes also help to prevent fungal invasion of tissues, and strawberries are gathered in early morning while the wax is still firm so that handling causes less damage.

Structure. The waxes are all *esters* of the **higher fatty acids** with **monohydric alcohols** of high molecular weight (p. 24). The acids which occur most commonly are **palmitic,** $C_{15}H_{31}\cdot COOH$, and **cerotic,** $C_{25}H_{51}\cdot COOH$, while the principal alcohols are **cetyl,** $C_{16}H_{33}OH$, **ceryl,** $C_{26}H_{53}OH$, and **melissyl** (myricyl), $C_{30}H_{61}OH$. Like the fats and oils, a mixture of esters occurs in one wax, and free fatty acids are often present as well. The waxes undergo hydrolysis less easily than the fats, but succumb when boiled for some time with alcoholic alkali. The alcohols may be extracted from the diluted solution with ether, and the acids may then be precipitated by acidification of the aqueous alkaline solution.

Properties. The waxes may be solid, semi-solid, or liquid. Only a few plant waxes have been investigated completely; some are isolated commercially, while others are by-products from different industries.

Carnaüba wax occurs as a thin coating on both the upper and lower surfaces of the leaves of the Palm, *Copernicia cerifera,* which grows in Brazil. It consists mainly of melissyl cerotate, $C_{25}H_{51}COOC_{30}H_{61}$, and contains free melissyl and ceryl alcohols, and also an isomer of lignoceric acid, $C_{23}H_{47}\cdot COOH$, sometimes called carnaübic acid. The wax is scraped off the leaves after they have been detached and sun-dried; it is then melted down and cast into blocks, and has various uses in the manufacture of candles, polishes, varnishes, etc.

Raphia wax occurs similarly on the leaves of the Raphia Palm (*Raphia Ruffia*); it is similar in composition to carnaüba wax, but contains in addition **arachyl alcohol,** $C_{20}H_{41}OH$.

Candelilla wax is obtained from several species (*e.g. Pedilanthus pavonis*) of the *Euphorbiaceæ* growing in New Mexico and Texas (U.S.A.). The shrubs are either steamed and the molten wax skimmed off, or they are extracted with solvents. This wax is used as a substitute for carnaüba wax, which it resembles.

Sugar-cane wax is obtained as a by-product in cane-sugar manufacture. The surface of the cane contains a hard wax which is extracted with solvents from the 'filter-press cake' after the sugar has been removed (p. 76).

Esparto wax has been prepared from the waste liquors in the manufacture of paper pulp from Esparto Grass (*Stipa tenacissima*). A similar product is obtained from the Australian 'cane' or 'bamboo' grass (*Glyceria ramigera*).

Poppy or **opium wax** obtained from the Opium Poppy (*Papaver somniferum*) consists mostly of ceryl palmitate and some free cerotic acid.

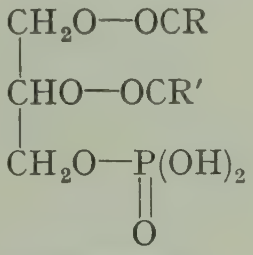
Pisang wax is obtained from the leaves of a variety of Banana tree (*Musa Cera*) which grows in Java ('Pisang' is the Malayan for 'banana'). It consists of an ester of an alcohol, $C_{13}H_{27}OH$ (which has been called **pisangceryl alcohol**) and an isomer of lignoceric acid (termed **pisangceric acid**).

Associated with the waxes in many plants are very small amounts of **hydrocarbons,** most of them of the saturated paraffin series (p. 8); *e.g.* $C_{30}H_{62}$ in the wax from the fruit of the Apple, $C_{32}H_{66}$ in candelilla wax, $C_{35}H_{72}$ in the wax from Fool's Parsley (*Æthusa Cynapium*); while from the leaf of the Cabbage (*Brassica oleracea*) the paraffin $C_{29}H_{60}$ and the corresponding ketone $(C_{14}H_{29})_2CO$ have been isolated. These are all solid substances at ordinary temperatures, and being insoluble in water, probably have a similar function to the waxes in forming a protective coating on the plant surfaces.

Compound Lipides (Lipins)

These substances, although present in very small amounts in plants, nevertheless occur in **all living cells,** both plant and animal. They are not storage materials, but are intimately connected with the cell metabolism. The term **lipin** covers substances of a fat-like nature which yield *fatty acids* on hydrolysis, and in addition contain *nitrogen*, or *phosphorus*, or both. There are two well-defined groups of lipins in plants: the **phosphatidic salts,** which are present in the actively growing tissues of plants, especially leaf and stem, in about equal proportion with the glycerides, and the **phospholipins,** which are found in the highest concentration in seeds. A third type, **galactolipins,** are characteristic of certain animal tissues, but comparable substances have been isolated from plants.

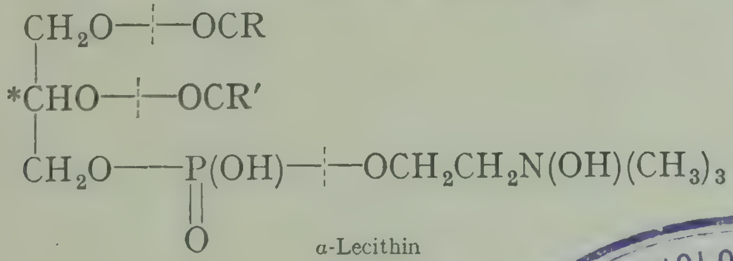
Phosphatidic Salts. In the phosphatidic salts, glycerol is esterified with only two molecules of fatty acids, and the third hydroxyl group is engaged in an ester linkage with phosphoric acid. This acidic group can then form metallic salts, and in plants the *calcium* salt occurs, with occasional substitution by magnesium. A phosphatidic acid was first obtained from the dried green leaves of Cabbage (*Brassica oleracea*) by extraction with ether and precipitation with acetone. The fatty acids comprise both saturated (palmitic) and unsaturated (linoleic, linolenic) members.



$$\begin{array}{l}\mathrm{CH_2O{-}OCR}\\ |\\ \mathrm{CHO{-}OCR'}\\ |\\ \mathrm{CH_2O{-}\underset{\underset{O}{\|}}{P}(OH)_2}\end{array}$$

Phosphatidic acid

Phospholipins. These compounds occur in relatively large amounts in some animal tissues, especially egg-yolk and brain and nerve tissue, and they are also universally distributed in plant cells, particularly in seeds of the *Leguminosæ*. They fall into three types: **lecithins, cephalins,** and **sphingomyelin.** The last mentioned apparently does not occur in plants. Lecithins are esters of the phosphatidic acids with an organic base, **choline,** $(CH_3)_3N(OH)CH_2{\cdot}CH_2OH$ (p. 129), the primary alcoholic group of the latter condensing with one of the acidic hydroxyl groups of phosphoric acid. Two formulæ for lecithin are therefore possible:



$$\begin{array}{l}\mathrm{CH_2O{-}\vdots{-}OCR}\\ \;|\\ \mathrm{{}^{*}CHO{-}\vdots{-}OCR'}\\ \;|\\ \mathrm{CH_2O{-}{-}\underset{\underset{O}{\|}}{P}(OH){-}\vdots{-}OCH_2CH_2N(OH)(CH_3)_3}\end{array}$$

α-Lecithin

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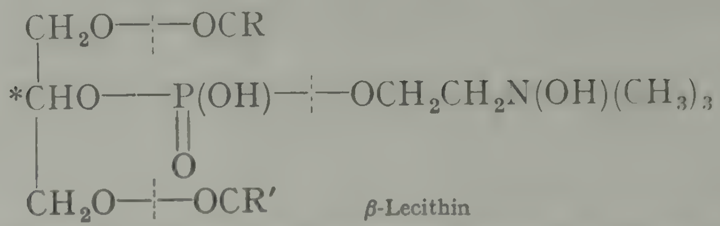


$$\begin{array}{l} CH_2O-\vdots-OCR \\ | \\ {}^*CHO---P(OH)-\vdots-OCH_2CH_2N(OH)(CH_3)_3 \\ | \qquad\qquad\quad \| \\ | \qquad\qquad\quad O \\ CH_2O-\vdots-OCR' \end{array}$$

β-Lecithin

Now naturally occurring lecithins on hydrolysis yield fatty acids, choline, and **glycerophosphoric acid,** and the latter is *optically active.* This can only happen when the asymmetric carbon atom (marked with an asterisk) in lecithin retains its asymmetry on hydrolysis. Therefore the α-form must be present. There is, however, the probability that the β-form also occurs along with the α-form in nature. Various lecithins can exist, depending on the acids concerned. So far, it appears that each lecithin always contains one saturated and one unsaturated acid residue; plant lecithins on hydrolysis give **stearic** and **palmitic** acids of the saturated group, and **oleic, linoleic,** and **linolenic** of the unsaturated, linoleic predominating in those studied. The characteristic acids of certain seed glycerides are also found in smaller amount in the phospholipins associated with them.

Cephalins appear to be associated with lecithins in all plant tissues. Chemically they differ from lecithin in containing the base **amino-ethyl alcohol** or **colamine** (p. 129), $CH_2(NH_2){\cdot}CH_2OH$, in place of choline. The base betaine (p. 127) also occurs associated with the plant lipins, and it may enter into their composition.

The phospholipins and their decomposition products have been obtained especially from seeds, but also from roots (*e.g.* Beetroot, Carrot, and Potato), and from leaves of Horse Chestnut (*Æsculus Hippocastanum*). The amount of phospholipin in seeds appears to vary with the protein content; thus the *Leguminosæ* contain relatively high proportions, *e.g.* the Soya Bean (*Glycine hispida*) contains 1·64 per cent. of its dry weight, whereas the cereals contain much smaller amounts, *e.g.* Maize (*Zea Mays*) contains only 0·28 per cent. This association of phospholipins with protein may be only a physical property, as both groups of compounds are colloidal and show strong adsorptive properties; but the possibility of chemical combination to give lecithoproteins (p. 149) is not ruled out, especially as the phospholipins, though soluble in ether, cannot be completely extracted from tissues with that solvent, but require additional treatment with alcohol. Phospholipins have been isolated by extraction with hot alcohol or ether-alcohol from many seeds, including the following:—

Leguminosæ: Lupin (*Lupinus luteus*), Soya Bean, Vetch (*Vicia sativa*), Broad Bean (*Vicia Faba*), Kidney Bean (*Phaseolus vulgaris*), Pea (*Pisum sativum*).

Gramineæ: Wheat (*Tricitum vulgare*), Oats (*Avena sativa*), Rye (*Secale cereale*), Barley (*Hordeum vulgare*), Maize.
Linaceæ: Flax (*Linum usitatissimum*).
Lecythidaceæ: Brazil nut (*Bertholletia excelsa*).
Malvaceæ: Cotton (*Gossypium*).

The plant phospholipins are yellowish-white, waxy substances which on exposure to air readily darken and absorb oxygen owing to the presence of unsaturated acids in the molecule. Hence their extraction and isolation in an unaltered state are difficult. They are soluble in most of the fat solvents, but are insoluble in acetone, which may be used to separate the phospholipins from the fats. The cephalins differ from the lecithins in being insoluble in cold alcohol, and therefore a separation of the two groups is made possible. The phospholipins are very hygroscopic, forming a plastic mass in the presence of moisture; in water they swell up and form either emulsions or colloidal solutions. They form double compounds with many metallic salts, especially cadmium chloride and platinum chloride, and this property is used in their purification. Since phospholipins contain a basic nitrogen atom, and also an acidic hydroxyl group in the phosphoric acid part of the molecule, they are amphoteric (p. 14). With alkali or mineral acids the phospholipins are hydrolysed to fatty acids, base, and glycerophosphoric acid, the latter being also partially decomposed to free phosphoric acid, especially on warming. *Lipase* can effect this hydrolysis at room temperature, and in addition there exists an enzyme, **phosphatase** or glycerophosphatase, in some seeds (*e.g.* of *Ricinus*) which hydrolyses glycerophosphoric acid to glycerol and phosphoric acid.

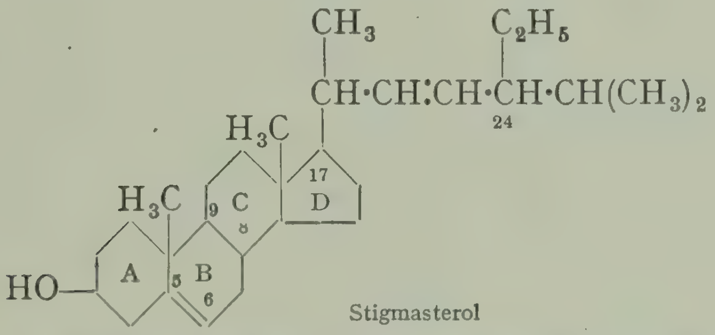
Galactolipins. The galactolipins are substances of a glycosidic nature (p. 64) containing *fatty acid*, *carbohydrate* (usually galactose), and a *base* in the molecule. Two well-defined compounds occur in brain tissue, and are therefore often called cerebrosides; **kerasin** contains lignoceric acid (p. 29), galactose, and the base **sphingosine,** $C_{18}H_{33}(OH)_2NH_2$, while in **phrenosin** lignoceric acid is replaced by the hydroxy-derivative of its next higher homologue, **phrenosinic acid,** $C_{24}H_{48}(OH)COOH$. A similar substance has been identified in Mushrooms (*Boletus edulis*) and in Rice (*Oryza sativa*). Much more important, however, is the fact that in the isolation of many of the plant phospholipins, carbohydrate also is found in the form of various sugars, especially glucose and galactose. For instance, Lupin seed and the grain of Wheat and Oats gave over 16 per cent. of sugars. Some of this sugar can be removed by repeatedly washing the extracts with water; and a physical complex of cephalin and glucose has been found in some animal tissues. But *part*, at least, of the carbohydrate appears to be in chemical combination, as it can only be removed after several hours' boiling with dilute mineral acid. Jamieson considers that the phosphatides are here present as glycosides (p. 64) rather than as galactolipins.

Physiological Function of Lipins. The lipins are associated with the metabolism of the cell, and thay have several possible functions. We have seen that they tend to form *adsorption complexes* especially with *proteins*, and the phospholipins in particular are related in structure not only to the fats, but also to the proteins. More important is the probability that they enter into the *structure* of the *cell membranes*, and hence **regulate** the **permeability** of the cell. Lecithins in contact with water have a remarkable property of appearing under the microscope to grow out with the formation of a large number of budding protrusions, the so-called *myelin* forms. This is due to the lowering of the surface tension of the water by the phosphoric acid and choline part of the molecule, and the consequent tendency to create as large a surface of contact as possible between the two. This is in direct contrast to the fats and oils, which tend to remain spherical—that is, with as small a common surface as possible; but resembles the action of the fatty acids, which form monomolecular films due to the polar carboxyl group (p. 13). Similarly lecithin, with its large polar group, *viz.* the cholyl-phosphoric acid group, which is soluble in water while the rest of the molecule is soluble in an oil phase, can form a *monomolecular film*. In this way a membrane, one or more molecules thick, may in fact be formed in the cell, though other substances in addition to the lipins may also take part. Such a membrane could confer on the cell the specific permeability to various ions and molecules which it is known to possess.

Sterols

When the crude fats and oils from animal or vegetable tissues are hydrolysed, a very small amount of ether-soluble material (usually not more than 0·5 per cent.) is not hydrolysed, and is therefore termed the **unsaponifiable residue.** It contains small amounts of hydrocarbons (p. 50) and related compounds, and the fat-soluble vitamins, but the major portion is composed of alcohols, known as sterols. These are **secondary alcohols** (since they give ketones on oxidation), and have a complex cyclic structure (cyclopentanoperhydrophenanthrene) based on the **phenanthrene** type nucleus (p. 226). The bile acids, the sex hormones, certain of the adrenal compounds, and the 'normal' cardiac aglucones (p. 107) have the same nucleus. Both saturated and unsaturated sterols occur, but the latter predominate. The plant sterols are grouped together as **phytosterols.** Few have been isolated in a pure condition, as several isomers are usually present, and these are difficult to separate. The sterols are colourless solids which can be crystallised from alcohol. They can be purified by condensation with digitonin (p. 109), followed by conversion into the acetates by boiling with acetic anhydride. Sterols occur in the free state, as esters of fatty acids, and as glycosides. Sterol glycosides or **phytosterolins** (Power and Salway) occur only in plants, and have been isolated from the bark of Olive and *Prunus*, from leaves of *Trifolium* species, and seeds of Watermelon.

Stigmasterol, $C_{29}H_{47}OH$, is widely distributed in plants both as the free sterol and as sterolins; Soya Bean and Calabar Bean oils contain comparatively large amounts. Stigmasterol can be converted into progesterone, and pharmaceutical preparations of this hormone are synthesised from stigmasterol from Soya Bean oil. Stigmasterol has the following formula:—



Stigmasterol

Unsaturated compounds with such a complex ring structure may exist in several isomeric forms, so it is not surprising that other sterols were found to be mixtures of isomers.

Sitosterols, $C_{29}H_{49}OH$. The sterol fraction from Wheat germ consists of a mixture of at least 5 sitosterols (α_1, α_2, α_3, β, and γ) and a dihydro-derivative, $C_{29}H_{51}OH$. The γ-form predominates; it is the most widely distributed sterol in plants. Soya Beans contain a mixture of the β- and γ-forms, the γ-form again predominating. These two forms are 22, 23-dihydro derivatives of stigmasterol, the isomerism being due in all probability to the configuration of the ethyl group on carbon atom number 24. β-Sitosterol occurs also in the Bean (*Phaseolus vulgaris*), and in Cinchona bark (where it was originally known as cinchol). It predominates, mixed with small amounts of α-sitosterol, in *Calycanthus* oil and in cottonseed oil. Sitosterol mixtures have also been isolated from the seeds of Rye and Maize and from the Calabar Bean (*Physostigma venenosum*).

Spinasterols, $C_{29}H_{47}OH$. Spinach contains three isomers, α-, β-, and γ-spinasterol, and the α-modification has also been isolated from Alfalfa. α-Spinasterol and zymosterol (from yeast) are the only known unsaturated sterols in which the double bond is not in the 5, 6 position; it is probably in the 8, 9 position. The side-chain of spinasterol is identical with that of stigmasterol.

Brassicasterol, $C_{28}H_{45}OH$, occurs in rape oil, and has a methyl group replacing the ethyl group on carbon atom number 24.

Fucosterol, $C_{29}H_{45}OH$, from Seaweed, is a dehydrostigmasterol and can be converted into it by catalytic hydrogenation.

Ergosterol, $C_{28}H_{43}OH$, was first isolated from ergot, the mycelia of the fungus *Claviceps purpurea*, which occurs on grasses and cereal crops, but especially on Rye. Ergosterol also occurs in yeast. It has two double bonds (5:6 and 7:8 in ring B), a third double bond in the side-chain (22:23 positions), and a methyl group replacing ethyl on carbon atom 24. Ergosterol has been very thoroughly investigated because by the action of ultraviolet radiation it is converted into several isomeric compounds, including **calciferol, vitamin D_2**. This is a fat-soluble, anti-rachitic vitamin similar to, but not identical with the naturally occurring vitamin D in fish liver oils. Several synthetic substances with anti-rachitic properties have been prepared, and it appears that fish liver oils contain more than one vitamin D. The chief anti-rachitic component is vitamin D_3, and its precursor in the sterols of the skin is 7-dehydrocholesterol (provitamin D_3), a derivative of cholesterol, the characteristic sterol of animal life. In **cholesterol,** $C_{27}H_{45}OH$, the side-chain is saturated, and carbon atom number 24 carries a second hydrogen atom in place of the ethyl group of stigmasterol.

Sterol Metabolism. The formation of sterols in both higher and lower plant forms can take place at any stage in their development, but the synthesis of sterols has been especially noted during the germination of seeds. During the germination of Soya Beans there is a diminution in the total fat content and an increase in sterol content.

PART III. ALDEHYDES, KETONES, AND CARBOHYDRATES

CHAPTER VII

ALDEHYDES AND KETONES

THE simple aldehydes and ketones do not occur in quantity in plants, but there is little doubt that they are actively concerned as intermediate products in plant metabolism. The significance of formaldehyde in relation to photosynthesis, of acetaldehyde and pyruvic aldehyde in respiration, is recognised. In addition, the simple sugars contain either aldehydic or ketonic groups, and owe many of their chemical reactions to this fact. Hence a study of the properties and reactions of these types must be undertaken first.

Structure

It has been shown (p. 21) that aldehydes are the products of the mild oxidation of primary alcohols and have the general formula R·CHO, while ketones are the oxidation products of secondary alcohols and conform to the formula R·CO·R′, where R and R′ may be the same or different organic radicals. The carbonyl group, $>C{=}O$, is common to both aldehydes and ketones. Aldehydes and ketones are found in all classes of organic compounds; individual aliphatic members will be discussed in this chapter. Aromatic and alicyclic members are also present in plants, *e.g.* benzaldehyde and salicylaldehyde (p. 181) are aromatic aldehydes, while the ketones menthone and camphor belong to the alicyclic or terpene group.

ALDEHYDES

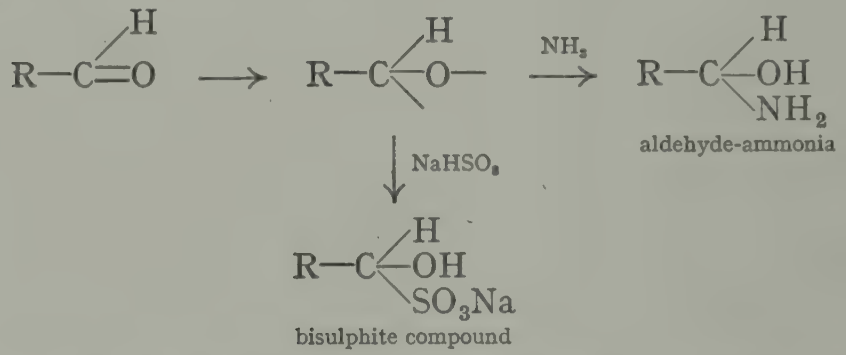
Chemical Reactions. Aldehydes generally show the following types of reaction:—

1. Aldehydes are good *reducing agents,* since they are easily oxidised to the corresponding acid (p. 21). They reduce an ammoniacal solution of silver nitrate (equivalent to a solution of silver oxide) to metallic silver, and they reduce Fehling's solution (equivalent to a solution of cupric hydroxide in Rochelle salt, p. 80) to an insoluble red precipitate of cuprous oxide. This

reaction with Fehling's solution is used in the estimation of the sugars.

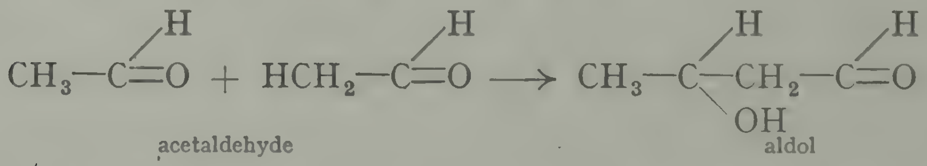
2. Aldehydes have a specific action on Schiff's reagent. This is a solution of the dye magenta (fuschine) decolorised with sulphur dioxide. Aldehydes regenerate the violet colour in the cold.

3. Aldehydes form *addition products*, the carbonyl group being converted to a hydroxyl group. Gaseous ammonia gives an **aldehyde-ammonia,** and as most of these derivatives are crystalline, they are used in the purification of aldehydes. Sodium bisulphite gives a crystalline **bisulphite compound,** and this is used in the isolation of aldehydes from the essential oils. The aldehydes can be regenerated in the former case by treatment with dilute acid, in the latter with warm alkali.



4. Aldehydes undergo *polymerisation*—that is, several molecules of the aldehyde become linked together to form a new molecule of multiple molecular weight. This takes place through the opening out of the double bond (*vide supra*), and the free valencies of several molecules link up to give the **polymer.** In some cases the original aldehyde can be regenerated (reversible polymerisation); in others, as in the formation of **aldehyde resins,** the change is irreversible.

5. Aldehydes form *condensation products*. The *aldol condensation* may be looked on as a special case of an irreversible polymerisation. Acetaldehyde and higher members of the aliphatic homologous series, $C_nH_{2n+1}CHO$, condense together in the presence of aqueous potassium carbonate to give compounds which contain both an aldehydic and an alcoholic group, *viz.* **aldols.**



This type of reaction probably occurs in nature, and provides a possible mechanism for the building up of long fatty-acid chains from the sugars or from aldehydic substances derived from the sugars (p. 288). Another important condensation product is the **phenylhydrazone,** formed by the action of phenylhydrazine. These phenylhydrazones are crystalline derivatives, and are especially used along with a similar derivative, the osazone, in the characterisation of the sugars (p. 67).

$$\underset{\text{aldehyde}}{R\text{—}CH}\boxed{O + H_2}\underset{\text{phenylhydrazine}}{N{\cdot}NH{\cdot}C_6H_5} \longrightarrow \underset{\text{aldehyde-phenylhydrazone}}{R{\cdot}CH{=}N{\cdot}NH{\cdot}C_6H_5} + H_2O$$

Formaldehyde

Formaldehyde, or Methanal, H·CHO, is a colourless gas with a pungent odour. It is very soluble in water, a 35–40 per cent. solution being known commercially as *formalin.* It is used as an insecticide and fungicide in the sterilisation of glasshouse soils; because of its hardening effect on the tissues, it is also employed in the preparation of anatomical specimens. Formaldehyde is usually prepared by the catalytic oxidation of methyl alcohol. It has reducing properties, develops the colour in Schiff's reagent, and forms several polymers. In the presence of lime-water formaldehyde polymerises to give a mixture of *sugars*, termed *formose,* and from this Emil Fischer isolated the simple sugar **fructose,** $C_6H_{12}O_6$. A similar polymerisation was postulated by Baeyer as occurring in photosynthesis. He assumed that the carbonic acid formed from the carbon dioxide and water was reduced to formaldehyde and that the latter polymerised to a sugar. The synthesis of formaldehyde in solutions of carbon dioxide has been obtained *in vitro* by various investigators, using catalysts such as magnesium and iron, and under the influence of sunlight and ultraviolet light. Baly and his school have shown that carbohydrates can be formed by irradiating solutions of carbon dioxide with visible light in conjunction with coloured absorbing substances such as nickel and cobalt salts. The detection of formaldehyde in the plant has proved the greater problem in this connection. Klein and Werner (1926) have established the presence of formaldehyde in actively assimilating leaves by identifying the known condensation product of formaldehyde with a cyclic compound, dimethyl*cyclo*hexanedione ('dimedone'). This condensation product is crystalline, and they showed that the reaction was only given by leaves which contained chlorophyll and which had been exposed to light. On the other hand, formaldehyde is also a decomposition product of other substances in the plant, especially on irradiation. In large amounts formaldehyde is toxic to the plant, and therefore *in vivo* instantaneous polymerisation of the formaldehyde to sugars must be postulated. It has also been suggested that formaldehyde never assumes the stable aldehydic form in the plant, but is transiently present as the more reactive hydroxyl form with the free valency (*v.s.*). Other investigators claim to have induced the synthesis of starch in leaves kept in the dark and supplied with formaldehyde vapour or solutions of formaldehyde instead of carbon dioxide; but there are many less toxic substances which the plant can absorb and from which it readily manufactures carbohydrates (*e.g.* mannitol). Hence there is little proof that formaldehyde is the *normal* intermediate product in photosynthesis.

EXPT. 16. *General Aldehyde Tests*

1. Add ammoniacal silver nitrate, and warm gently in a water-bath if necessary. A silver mirror is produced.

2. Add Fehling's solution and boil. A red precipitate of cuprous oxide is formed.

3. Add a few drops of Schiff's reagent, when a violet colour is developed in the cold.

4. Shake with a saturated solution of sodium bisulphite; a crystalline deposit of the bisulphite compound separates.

Formaldehyde

1. Show that a solution of formaldehyde (formalin) gives the above general aldehyde tests.

2. Evaporate 1 c.c. of formalin on a watch-glass in a water-bath. A solid mass of paraformaldehyde remains. Heat this in a test-tube, when formaldehyde will be re-formed and evolved as a gas.

Acetaldehyde

Acetaldehyde, or Ethanal, $CH_3 \cdot CHO$, is a colourless liquid, b.p. 21° C., with a characteristic, sharp odour. It may be prepared by the oxidation of ethyl alcohol with chromic acid. It is the first typical member of this homologous series, giving all the general reactions of aldehydes. Acetaldehyde occurs in small amounts in many plant tissues, especially in ripe fruits. Its appearance there in the tannin sacs of the mesocarp is concomitant with, if not responsible for, the coagulation of the tannin and the disappearance of the astringent taste. Acetaldehyde has been shown by its removal as the bisulphite compound or as the 'dimedone' condensation product to be one of the intermediate products in the fermentation of sugars (p. 257) and also in plant respiration (p. 281). In anaerobic respiration of apples, induced by lack of oxygen, acetaldehyde accumulates together with ethyl alcohol, giving 'brown heart,' a physiological breakdown which sometimes occurs in storage (p. 315).

EXPT. 17. *Acetaldehyde*

1. Show that acetaldehyde gives the general aldehyde tests.

2. Add carefully one drop of concentrated sulphuric acid to a little acetaldehyde in a test-tube. On dilution with water, paraldehyde separates as an oil. On heating this, acetaldehyde is re-formed.

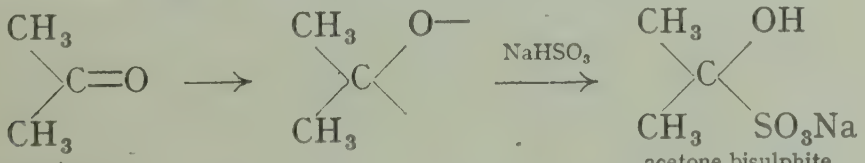
3. Warm some acetaldehyde with aqueous sodium hydroxide. A yellow or brown-red resin is formed.

Higher aldehydes of this series are present in very small amounts in plants, and are isolated in the essential oils. Of the *unsaturated* aldehydes, **acrolein,** $CH_2{=}CH{\cdot}CHO$, is the simplest. It is formed by heating glycerol with potassium bisulphate, and may be used as a test for the former (p. 25). A higher homologue, αβ-**hexylene aldehyde,** $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH{=}CH{\cdot}CHO$, has been isolated from the leaves of the Hornbeam (*Carpinus*). **Citronellal,** $C_{10}H_{18}O$, with one double bond, and **citral,** $C_{10}H_{16}O$, with two, occur in essential oils and are discussed with the terpenes, to which they are closely related (p. 237).

α-**Glyceric aldehyde,** $CH_2OH{\cdot}CHOH{\cdot}CHO$, is an aldehyde derived structurally from glycerol (p. 24). This aldehyde and the related acid, α-**glyceric acid,** $CH_2OH{\cdot}CHOH{\cdot}COOH$, are intermediate compounds in fermentation and in respiration (pp. 256 and 281).

KETONES

The simplest aliphatic ketone is **acetone,** or dimethyl ketone, $CH_3{\cdot}CO{\cdot}CH_3$. It occurs in small amounts in pyroligneous acid (p. 21), and it is also obtained by the decomposition of starch by a bacterial preparation known as 'Fernbach's culture.' Fermentation of grain, potatoes, and molasses with *B. Clostridium acetobutylicum* (Weizmann) gives *n*-butyl alcohol (60 per cent.), acetone (30 per cent.) and ethyl alcohol (10 per cent.). Acetone is a colourless liquid with a pleasant, though sharp smell. It is a



acetone   acetone bisulphite

$$\underset{\text{acetone}}{(CH_3)_2CO} + \underset{\text{phenylhydrazine}}{H_2N{\cdot}NH{\cdot}C_6H_5} \longrightarrow \underset{\text{acetone phenylhydrazone}}{(CH_3)_2C{=}N{\cdot}NH{\cdot}C_6H_5} + H_2O$$

representative ketone and differs from aldehydes in that (*a*) it shows no reducing action on ammoniacal silver nitrate or on

Fehling's solution, (*b*) it has no effect on Schiff's reagent in the cold, and (*c*) it does not polymerise, giving, for instance, no resin on being warmed with alkali. Ketones, however, resemble aldehydes in giving a **bisulphite addition product,** and this is used in the extraction of some of the ketonic as well as aldehydic constituents of essential oils. Ketones also give condensation reactions with phenylhydrazine, forming **phenylhydrazones.**

EXPT. 18. *Acetone*

Show that acetone does not give the first three general aldehyde tests (a silver mirror may be produced on prolonged boiling), but that it gives a crystalline bisulphite compound. Show that acetone gives no resin on warming with sodium hydroxide solution.

CHAPTER VIII

CARBOHYDRATES. SUGARS

CLASSIFICATION

THE carbohydrates are of fundamental importance as the primary products of photosynthesis in plants, and all complete theories as to the building up of other organic substances in the plant must start from the carbohydrate unit. Carbohydrates are also involved in the maintenance and growth of the living plant, as they not only form the main substrate for respiration, but also act as one of the storage forms in seeds, roots, and tubers, and comprise the framework of the plant cell-walls.

The carbohydrates derive their name from the fact that most of them can be represented by the general formula $C_x(H_2O)_y$—that is, they contain carbon, hydrogen, and oxygen, the last two elements being present in the proportions in which they occur in water. These include the **sugars, starches,** and **celluloses.** Related substances are the *hemicelluloses*, *pectins*, *gums*, and *mucilages*, which usually contain an acidic part in addition to the sugar part of the molecule. The simpler members of the carbohydrates are the sugars, which are crystalline solids, sweet to the taste, and soluble in water. Most of the natural sugars contain either six or twelve carbon atoms in the molecule; the former are taken as the unit and called **monosaccharides,** and are represented by the formula $C_6H_{12}O_6$, while the twelve-carbon sugars are the **disaccharides,** $C_{12}H_{22}O_{11}$. A few sugars of greater molecular complexity, the **tri-** and **tetra-saccharides,** also occur in plants. Still more complex are the **polysaccharides,** which include the starches and celluloses. These are usually amorphous, tasteless, and either insoluble in water or capable of forming only colloidal solutions.

The relationship between these types of carbohydrates is seen on *hydrolysis*. The disaccharides give two molecules of monosaccharides according to the equation:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow 2C_6H_{12}O_6$$

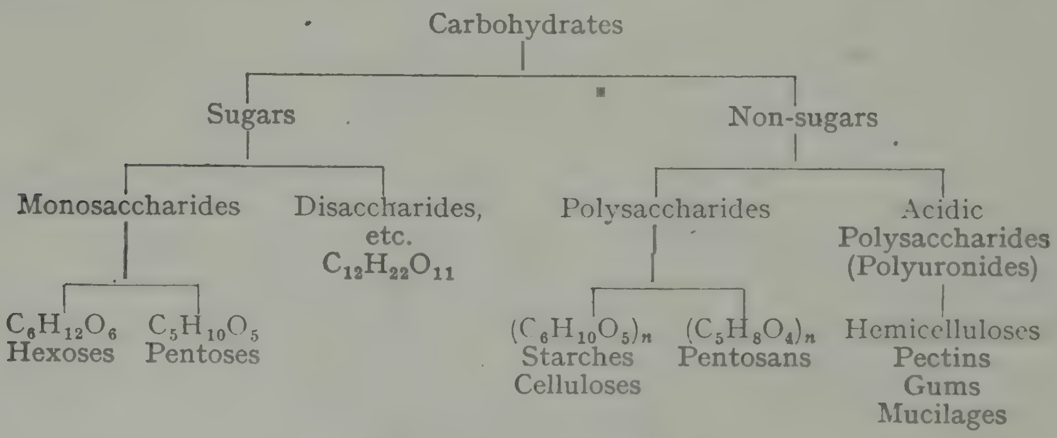
Similarly, the starches and celluloses, which conform to the general formula $(C_6H_{10}O_5)_n$, can also be hydrolysed to monosaccharides:

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6$$

Another type of polysaccharide occurring in plants and called a **pentosan** is hydrolysed to a sugar containing five carbon atoms:

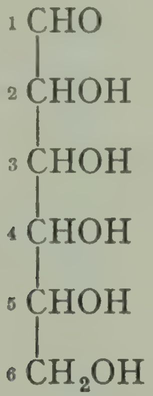
$$(C_5H_8O_4)_n + nH_2O \longrightarrow nC_5H_{10}O_5$$

Hence the monosaccharides are subdivided into C_6-sugars or **hexoses,** and C_5-sugars or **pentoses.** Other monosaccharides containing from two to nine carbon atoms are known, but only two or three occur naturally. Finally, there are the polysaccharides containing acidic groupings, such as the hemicelluloses and the pectins; the name **polyuronides** has been suggested for these. This chemical classification of the carbohydrates is paralleled to some extent by the *function* of the different types in the plant. The sugars, which will be discussed in this chapter, are metabolic products in solution in the cell-sap; they also act as storage materials in some roots and bulbs, *e.g.* Carrot, Turnip, Mangold, Onion, etc. Of the polysaccharides, the starches act as reserve or storage materials in seeds and tubers, *e.g.* cereal grains and Potato and Dahlia tubers, while the celluloses and their associated substances, the hemicelluloses and pectins, form the 'framework' of the plant. These will be dealt with in succeeding chapters. Finally, many of the sugars occur combined with other substances in plants, forming the plant pigments, the tannins, and many other compounds. These are all grouped together under the term **glycosides** (Armstrong). A glycoside may be defined as a substance which furnishes a sugar or sugars and one or more other products on hydrolysis. Some of these sugars are rare, only occurring in one or two glycosides, and not in any other natural products yet investigated. The following table summarises the classification of the carbohydrates outlined above:—


Carbohydrates
Sugars
Non-sugars
Monosaccharides
Disaccharides, etc. $C_{12}H_{22}O_{11}$
Polysaccharides
Acidic Polysaccharides (Polyuronides)
$C_6H_{12}O_6$ Hexoses
$C_5H_{10}O_5$ Pentoses
$(C_6H_{10}O_5)_n$ Starches Celluloses
$(C_5H_8O_4)_n$ Pentosans
Hemicelluloses Pectins Gums Mucilages

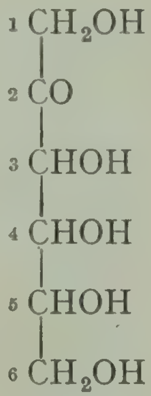
MONOSACCHARIDES

Structure. The monosaccharides are related to the 'straight-chain' polyhydric alcohols (p. 25) in that the hexoses can be reduced to the hexahydric alcohols, and the pentoses to the corresponding pentahydric compounds. The monosaccharides contain *hydroxyl* groups, and in addition some contain an *aldehyde* (—CHO) and others a *ketone* (·Ċ:O) group. Glucose and fructose are the typical members, glucose being an **aldohexose** (I), and fructose a **ketohexose** (II).



$$\begin{array}{l} {}_1\,CHO \\ \;\;| \\ {}_2\,CHOH \\ \;\;| \\ {}_3\,CHOH \\ \;\;| \\ {}_4\,CHOH \\ \;\;| \\ {}_5\,CHOH \\ \;\;| \\ {}_6\,CH_2OH \end{array}$$

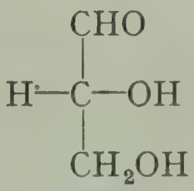
(I)
Aldohexose



$$\begin{array}{l} {}_1\,CH_2OH \\ \;\;| \\ {}_2\,CO \\ \;\;| \\ {}_3\,CHOH \\ \;\;| \\ {}_4\,CHOH \\ \;\;| \\ {}_5\,CHOH \\ \;\;| \\ {}_6\,CH_2OH \end{array}$$

(II)
Ketohexose

Isomerism in each type is possible, depending on the relative arrangement of the hydrogen atoms and hydroxyl groups round the carbon atoms. Thus galactose and mannose are also aldohexoses, but differ from glucose in the arrangement of these groups (*cf.* formulæ (VII), (IX), and (XI)). The configurational relationships among the sugars is indicated by the use of the symbols *D*- and *L*-. A sugar belongs to the *D*-series when the hydroxyl group on the carbon atom adjacent to the primary alcoholic group is on the right (Rosanoff convention). The simplest reference compound is *D*-α-glyceric aldehyde, as follows:—



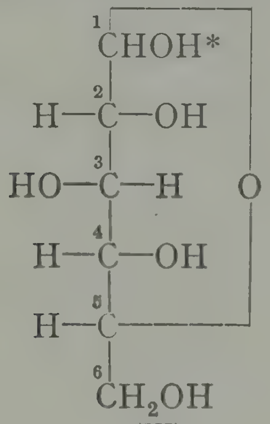
$$\begin{array}{c} CHO \\ | \\ H—C—OH \\ | \\ CH_2OH \end{array}$$

D-Series

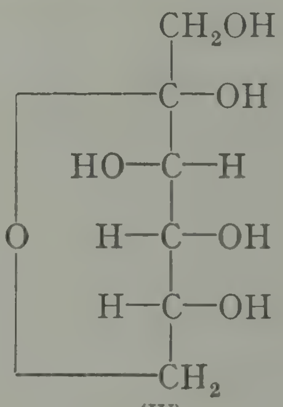
A similar type of isomerism due to spatial arrangement is important in the amino-acids (p. 132).

Also, since these molecules contain *asymmetric carbon atoms* (*viz.* carbon atoms 2, 3, 4, 5 in glucose (I)), optical isomers are possible. The naturally occurring carbohydrates are all optically active, most of these being the *dextro*-modifications. Fructose, which is *lævo*-rotatory, is the most important exception.

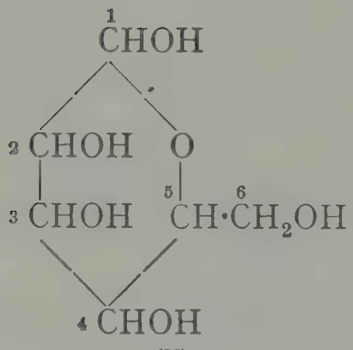
That the above 'straight-chain' formulæ do not in all cases satisfactorily represent the sugar molecule is shown by several reactions; for instance, (*a*) these sugars do not exhibit all the properties of aliphatic aldehydes or ketones, *e.g.* they do not form bisulphite compounds; (*b*) in the formation of the monomethyl ether of glucose *two* **methyl glucosides** are obtained. Glucose itself has also been shown to exist in two modifications, α- and β-glucose, and this can only occur if carbon atom number one (1) is asymmetric. The explanation is that ring formation takes place between the aldehydic or ketonic group and one of the hydroxyl groups. The **six-membered** or *amylene oxide* ring is the most stable arrangement; the cyclic formula for glucose may therefore be written as in formula (III), and the α- and β-forms depend on the relative arrangement about the ring of the hydrogen and hydroxyl group on carbon number one. Further, α- and β-methyl glucosides are possible, and similar derivatives of α- and β-glucose occur in the disaccharides, the polysaccharides, and in the glucosides. In all these cases the asterisked hydrogen (III) is replaced by another group (CH_3 in the methyl-glucosides), and the relative position of this group to the other groups arranged about the plane of the ring determines whether the compound is a derivative of α- or β-glucose.



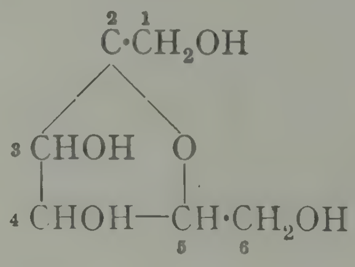
(III)
Glucose



(IV)
Fructose



(V)
Gluco-, galacto-, etc.,
-pyranose

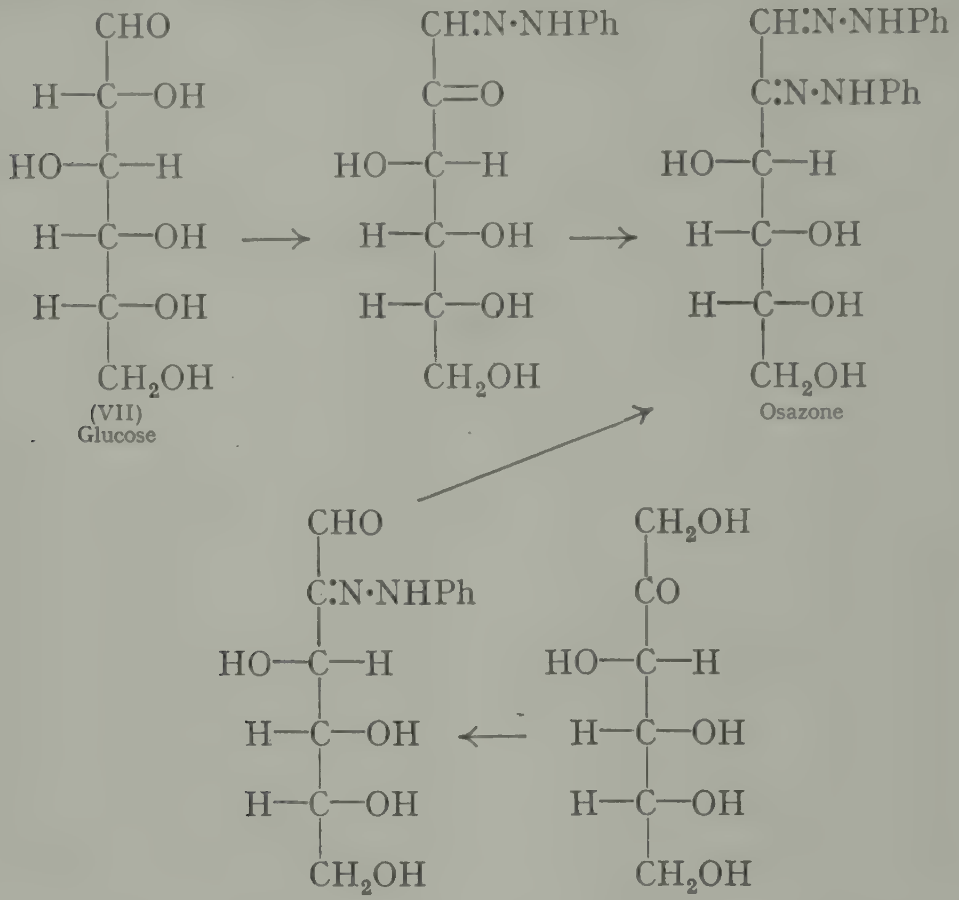


(VI)
Fructofuranose

The fructose molecule also possesses the amylene oxide ring as shown in formula (IV). This six-membered ring structure for the sugars is related to the heterocyclic *pyran* ring (*cf.* pyrone, p. 6), and therefore Haworth suggests the terminology **glucopyranose** and **fructopyranose** for formulæ (III) and (IV) respectively. Formula (V) is another way of writing the general cyclic formula for the pyranose aldohexoses.

That a type of ring structure other than the normal form could exist was first shown in the case of glucose by Emil Fischer, and it was called the γ-form. This was very reactive, and could only exist as derivatives, the free sugar immediately reverting to the normal structure. Later it was found that the **fructose** molecule present in the naturally occurring disaccharide *sucrose* and in the polysaccharide *inulin* was a γ-sugar. Hydrolysis of both these substances gives normal fructopyranose (2 : 6 ring), but if the free hydroxyl groups be methylated before hydrolysis, the fructose part appears as a derivative of a five-membered (2 : 5) or **furanose** sugar (related to *furan*), formula (VI). This method of methylation and subsequent hydrolysis—evolved by Purdie, Irvine, and their collaborators—enables not only the cyclic structures of the constituent sugars to be determined, but indicates also the points of union of these units. It has therefore made possible the elucidation of the structure of the disaccharides, and to some extent also that of the polysaccharides.

Properties and General Reactions. The monosaccharides are crystalline substances, sweet and soluble in water. They cannot be hydrolysed to simpler carbohydrates. Many of their reactions can be explained on the open-chain structural formulæ. Both the aldehyde and ketone groups in sugars are easily oxidised, and therefore the monosaccharides reduce an ammoniacal silver nitrate solution and Fehling's solution. They also give a yellow and then brown coloration on being heated with alkali, owing to the formation of resinous products. They form additive compounds, especially with phenylhydrazine. Three molecules of the latter are concerned, one forming the phenylhydrazone on the aldehyde or ketone group, the second oxidising the adjacent alcoholic group (in glucose to a ketone group in position 2, in fructose to an aldehyde in position 1), and the third molecule condensing with the new group. The resulting compounds, called **osazones,** are yellow, relatively insoluble solids with sharp melting-points and well-defined crystalline structure under the microscope. They are therefore useful in characterising the sugars. It will be seen from the following formulæ that glucose (VII) and fructose (VIII) have the same osazone, since the arrangement in space of the atoms in positions 3, 4, 5 is identical.

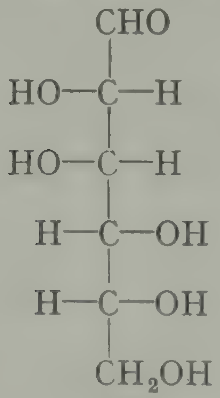


(VII) Glucose

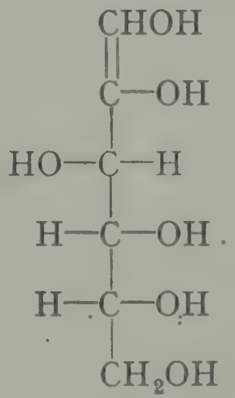
Osazone

(VIII) Fructose

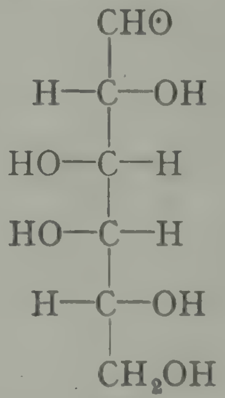
This similarity of structure is shown further in the fermentation of sugars to alcohol by yeast. Glucose, fructose, and mannose (IX) are all easily fermented, whereas galactose (XI) is not; the explanation is that the three fermentable sugars act through the common **enolic form** (X), which is not the same as that derivable from galactose, owing to the different arrangement in position 4.



(IX) Mannose



(X) Enol of glucose, fructose, mannose

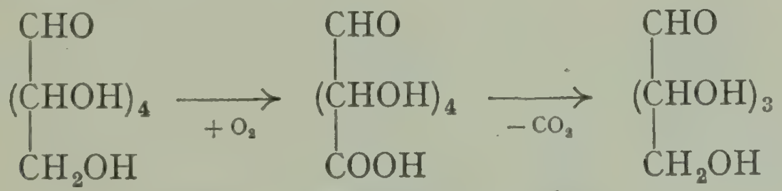


(XI) Galactose

That the enol form is the intermediate product is indicated by the fact that in fermentation and in the parallel first stage of the respiration process, a hexose diphosphate which is a fructose derivative is formed, no matter which of these three sugars is the substrate. The interconversion of glucose, fructose, and mannose can be accomplished in the laboratory by the action of dilute alkali.

Uronic Acids. We have seen that the monosaccharides are easily oxidised to acids; but different acids are obtained under different experimental conditions. The most important acids from the biochemical viewpoint are those derived from glucose, galactose, and mannose, in which the terminal primary alcoholic group (position 6) is oxidised. These acids, **glucuronic, galacturonic** and **mannuronic acids,** generally termed the **uronic acids,** occur in polymerised forms in the pectins and other acid polysaccharides, hence the term polyuronides. The uronic acids also in a few instances replace sugars in the naturally occurring glycosides.

Decarboxylation, or the loss of carbon dioxide from a carboxylic acid group is a common reaction in plants, for they possess an enzyme called *carboxylase* which acts as catalyst. Hence we have a mechanism for the synthesis in the plant of **xylose** and **arabinose,** the pentoses most widely distributed in plants in the pentosans, gums, and related substances. Xylose and arabinose have not only the same configuration as glucose and galactose respectively in positions 2, 3, and 4, but they retain the six-membered ring or pyranose structure.



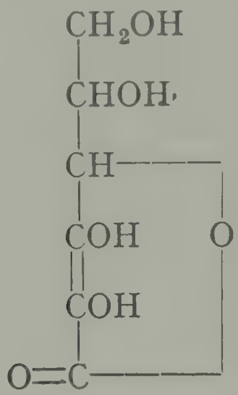
$$\begin{array}{c} \mathrm{CHO} \\ | \\ (\mathrm{CHOH})_4 \\ | \\ \mathrm{CH_2OH} \end{array} \xrightarrow[+\,O_2]{} \begin{array}{c} \mathrm{CHO} \\ | \\ (\mathrm{CHOH})_4 \\ | \\ \mathrm{COOH} \end{array} \xrightarrow[-\,CO_2]{} \begin{array}{c} \mathrm{CHO} \\ | \\ (\mathrm{CHOH})_3 \\ | \\ \mathrm{CH_2OH} \end{array}$$

glucose ⟶ glucuronic acid ⟶ xylose
galactose ⟶ galacturonic acid ⟶ arabinose

This derivation of the pentoses from hexoses is borne out by the fact that in plant tissues glucose and xylose are frequently associated in glucosides, while glucose, glucuronic acid, and xylose occur in complex polysaccharides. Also, the shoots of the Bamboo (*Bambusa*) contain in the cell-sap xylose and glucuronic acid. Similarly, galactose, galacturonic acid, and arabinose are associated, especially in pectins and gums and in the hemicelluloses of wood.

A substance related to the uronic acids and originally termed *hexuronic acid* by its discoverer, Szent-Györgyi, is a possible catalyst in respiration (p. 285) and has also been identified with **vitamin C.**

It has been synthesised by Haworth, Hirst, and their collaborators (1933), and is the **lactone** of the enol form of a keto-uronic acid. (A lactone is formed by the elimination of water between an acid and a hydroxyl group in the same molecule.) It is now known as *L*(+)-ascorbic acid. It is present in most plants, and reaches high concentrations in *Citrus* fruits and in berries.



Ascorbic acid, or hexuronic acid

Hexoses

Three aldohexoses—glucose, galactose, and mannose—are common in plants, either in the free state or as units of the polysaccharide molecules, while only one ketohexose occurs, namely, fructose.

Glucose, *Dextrose,* or *Grape Sugar,* occurs in all plants, especially in *green leaves* along with fructose and sucrose. It is present in *ripe fruits,* including grapes, and also as storage material in many *roots, tubers,* and *bulbs, e.g.* Carrot, Onion, and in some *seeds, e.g.* Almond, Sweet Chestnut. It is by far the most common sugar in the large class of glycosides, and is the unit from which the molecules of starch and cellulose are built. It is prepared commercially by the hydrolysis of starch with dilute mineral acid, and is used in the form of a thick syrup in confectionery and fruit-preserving. When pure, glucose is a colourless crystalline solid, less sweet than cane sugar. It is *dextro*-rotatory, hence its alternative name, dextrose. Glucose may be the first sugar formed by the plant in the photosynthetic process; a discussion of this is left to a later chapter (p. 276).

EXPT. 19. *General Test for Carbohydrate*

Molisch's Test. Show that aqueous solutions of glucose, sucrose, maltose, and starch (p. 12), on shaking with a few drops of a 1 per cent. solution of *a*-naphthol in alcohol, give a violet coloration when concentrated sulphuric acid is poured carefully down the inside of the test-tube.

EXPT. 20. *Hexoses: Examination of Glucose*

1. Show that a solution of glucose turns yellow, then reddish-brown, on being warmed with sodium hydroxide solution.

2. Show that on warming a glucose solution with Fehling's solution, the latter is reduced, and red cuprous oxide is precipitated.

3. Preparation of glucosazone. Dissolve a little glucose in 10 c.c. of water in a test-tube, add a few crystals of phenylhydrazine hydrochloride, twice that amount of solid sodium acetate, and a few drops of acetic acid. Place the test-tube in a beaker of boiling water for 15 minutes, then cool the test-tube under the tap. The osazone separates as a mass of yellow crystals.

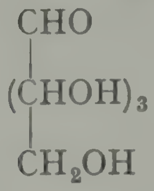
Similar tests may be applied to other hexoses.

Fructose, *Lævulose,* or *Fruit Sugar,* occurs with glucose in the green parts of plants, in sweet fruits, and in some roots and bulbs. It also occurs condensed as the polysaccharide *inulin,* the storage material in some roots and tubers. It may be prepared by the hydrolysis of inulin with dilute acid. Alike in inulin, in the disaccharide *sucrose,* and in the trisaccharides *raffinose* and *gentianose,* it occurs as the **furanose** or γ-form, but on hydrolysis the free fructose obtained has the normal pyranose structure. Fructose occurs in a few glycosides only, mainly the saponins. Fructose is relatively difficult to crystallise; it has a sweet taste and is *lævo*-rotatory, hence the name lævulose.

Galactose does not normally occur free in plants, although an abnormal production of galactose on berries of the Ivy due to frost has been recorded (p. 318). Galactose is a constituent of the trisaccharide raffinose, the tetrasaccharide stachyose, and is present in many gums, mucilages, and pectins. Several galactosides occur in the saponin group of glycosides and in the anthocyanin pigments. Galactose also occurs combined with glucose in the disaccharide lactose, the typical animal sugar which does not occur in plants. Galactose is a sweet, crystalline substance, *dextro*-rotatory, and an aldohexose; it gives a different osazone from glucose.

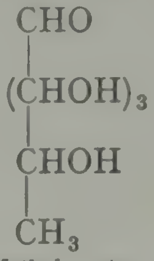
Mannose is another *dextro*-rotatory aldohexose, and gives the same osazone as glucose and fructose. It occurs in plants in the form of *mannans,* in various roots and seeds. Its main source is 'vegetable ivory,' the endosperm of the nut of the Tagua Palm (*Phytelephas macrocarpa*), from which it is prepared by hydrolysis with acid. These mannans also occur as the secondary thickening in the cell-wall of the endosperm of the Date (*Phœnix dactylifera*), and in the Brazil Nut (*Bertholletia excelsa*).

Pentoses and Methyl-pentoses



$$\begin{array}{l} CHO \\ | \\ (CHOH)_3 \\ | \\ CH_2OH \end{array}$$

Pentose



$$\begin{array}{l} CHO \\ | \\ (CHOH)_3 \\ | \\ CHOH \\ | \\ CH_3 \end{array}$$

Methyl-pentose

The pentoses occur in nature as the polysaccharide *pentosans* in straw, bran, and woody tissues. They also form part of the molecule of the plant gums, mucilages, and pectins, whereas the methyl-pentoses are mostly found in the glycosides. The most general reaction of the pentoses is the formation on warming with mineral acids of **furfural,** a heterocyclic aldehyde, which gives a bright red coloration with aniline, and coloured condensation products with phenols; thus, phloroglucinol is used in the estimation of pentoses. The pentosans give similar results, the acid first hydrolysing them to pentoses.

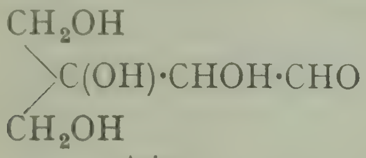
Four aldopentoses are possible, each in two optically active modifications. The two active forms of **arabinose** occur naturally, and so do the *dextro*-rotatory forms of **xylose** and **ribose.** These differ, like the aldohexoses, in the distribution of the hydroxyl groups round the asymmetric carbon atoms. The system of naming the optical isomers of the sugars by the use of the prefixes *D*- and *L*- is derived from their relation to the configuration of glucose, and in some cases this does not correspond with the sign of rotation of the substance in question. Arabinose is a case in point: the common form in nature is *dextro*-rotatory, but because it has the same configuration as *L*-glucose, it is written *L*(+)-arabinose.

Arabinose is obtained as the *dextro*-rotatory modification (*L*(+)-arabinose) principally by the hydrolysis of **gum arabic** and **cherry gum**; it also occurs in other gums, many mucilages, and in some complex glycosides. The *lævo*-rotatory form, *L*(−)-arabinose, occurs in the glycoside **barbaloin** from species of *Aloe*.

D(+)-**Xylose** is obtained from the pentosan **xylan** and various polysaccharides in woody tissues, straw, bran, wood gum, and the shells of some seeds. It also occurs in the disaccharide *primeverose*.

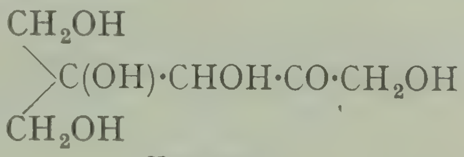
D(+)-**Ribose** is the unique sugar of one group of **nucleic acids** (p. 166); like fructose, it possesses a furanose ring structure. The other sugar obtained from nucleic acids is **desoxyribose,** $CH_2OH \cdot CH_2 \cdot CHOH \cdot CHOH \cdot CHO$.

A curious pentose, termed **apiose,** occurs as the glycoside **apiin** in Parsley (*Petroselinum sativum*). It contains a *branched* chain, being structurally related to *iso*valeric acid. The only other branched-chain sugar so far found in nature is **hamamelose,** a ketohexose combined in **hamameli tannin.**



$$\begin{matrix}CH_2OH\\ \\ CH_2OH\end{matrix}\!\!>C(OH)\cdot CHOH\cdot CHO$$

Apiose



$$\begin{matrix}CH_2OH\\ \\ CH_2OH\end{matrix}\!\!>C(OH)\cdot CHOH\cdot CO\cdot CH_2OH$$

Hamamelose

The **methyl-pentoses** are derivatives of the pentoses, in which one of the hydrogens of the terminal primary alcoholic group has been replaced by the methyl radical. Four methyl-pentoses occur in plants.

Rhamnose is the most widely distributed methyl-pentose. It occurs with glucose as complex glycosides in the **anthoxanthin** and **anthocyanin plant pigments,** and also in other glycosides as the disaccharide *rutinose* and the trisaccharides *robinose, rhamninose, solanose,* etc. **Epirhamnose** (or epirhodeose) is an isomeric methyl-pentose occurring as a glycoside in the bark of species of *Cinchona,* and in the saponin glycosides of the *Convolvulaceæ* along with *d*-**fucose** (or rhodeose). *l*-**Fucose,** the optical antipode, occurs in the pentosan **fucosan,** which is present in the cell-wall structure of many seaweeds. Several rare pentose sugars occur in the *Digitalis* and *Strophanthin* glycosides: **digitoxose,** $CH_3\cdot(CHOH)_3\cdot CH_2\cdot CHO$, is a 2, 6-bisdesoxyhexose, and **cymarose** is its 3-methyl ether. **Sarmentose** and **digitalose** form a similar pair, sarmentose being 6-desoxy-*d*-galactose.

EXPT. 21. *Pentoses: Examination of Arabinose*

1. Show that a solution of arabinose reduces Fehling's solution.

2. Show that on warming an arabinose solution with alkali a yellow or brown colour is developed.

3. Heat a few c.c. of a solution of arabinose with half its volume of concentrated hydrochloric acid in a test-tube, and hold a piece of filter-paper soaked in aniline acetate solution near the mouth of the test-tube. A red colour is developed on the paper.

4. Warm an arabinose solution with an equal volume of concentrated hydrochloric acid, and add a small quantity of phloro-glucinol. A bright red colour is produced.

5. Show that arabinose forms an osazone.

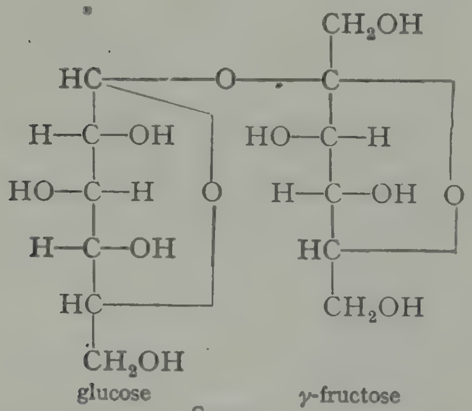
Other Monosaccharides

Triose. A three-carbon sugar-like compound with the formula $CH_2OH\cdot CHOH\cdot O\cdot CH_2OH$ has been isolated from leaves of the Cabbage.

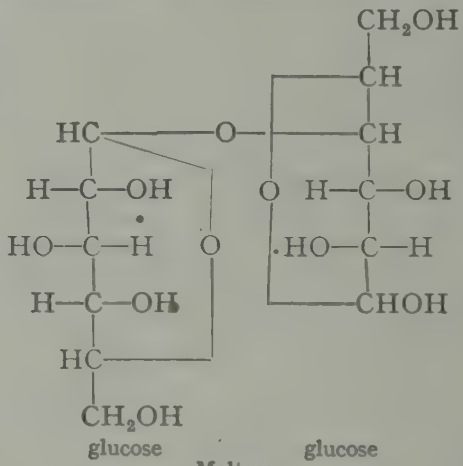
Heptoses. Two straight-chain monosaccharides containing seven carbon atoms have been isolated from plants. **Mannoketoheptose** occurs in the Avocado Pear with the corresponding alcohol, perseitol, while **sedoketoheptose** occurs in the Stonecrop (*Sedum spectabile*).

DISACCHARIDES

Structure. The disaccharides are condensation products of two monosaccharide molecules with the elimination of one molecule of water. Hydrolysis with dilute acid, or in many cases by an enzyme, regenerates the constituent monosaccharides. The disaccharides are related to the methyl glucosides: one of the monosaccharide units is linked through the hydroxyl from the aldehyde or keto-group (*cf.* formulæ (III) and (IV), p. 66) to a hydroxyl in the second molecule, the ring structures of each molecule being retained. If this second hydroxyl is also derived from the reducing group of its sugar, then the disaccharide molecule is *non-reducing*, as is the case with sucrose. But usually one of the other hydroxyls is involved, and the disaccharide has a definite reducing power, which, however, will be less than that of its constituent molecules. Also, two possibilities always exist corresponding to the α- and β-modifications of the methyl glucosides. Where glucose itself is condensed through the aldehydic group, the action of enzymes serves in many cases to discriminate between α- and β-glucosides; thus, *maltase* from yeast hydrolyses α-glucosides, and *emulsin* from almonds acts on β-glucosides. The methods for determining the structure of the disaccharides include the methylation method (p. 67), a method of oxidation and identification of the products (Haworth), and Zemplen's degradation method, whereby the chain of carbon atoms in the disaccharide is shortened step by step until the position of union of the units is reached. The disaccharides



Sucrose



Maltose

which occur *free* in plants and animals all contain two hexose units; in plants several rare disaccharides occur condensed with other residues to form more complex glycosides. In such cases the free reducing group (of the sugar listed first in the following table) is condensed with a hydroxyl group in the non-sugar part of the molecule. Almost all the disaccharides contain glucose as one of the constituent monosaccharides. The structural formula of sucrose is appended, although it has not yet been definitely established whether the α- or β-forms of the monosaccharide units are involved. There follows a list of plant disaccharides with their structure so far as it has been determined (using the numbering of formulæ (III) and (VI)), and their source if not occurring in the plant in the free state:—

Sucrose . . .	2-γ-fructose-α-glucose	
Turanose . . .	6-γ-fructose-α-glucose	Trisaccharide melicitose.
Maltose . . .	4-glucose-α-glucose	Polysaccharide starch.
Cellobiose . . .	4-glucose-β-glucose	Polysaccharide cellulose.
Gentiobiose . . .	6-glucose-β-glucose	Trisaccharide gentianose, glycoside amygdalin.
Trehalose . . .	1-α-glucose-α-glucose	
[Lactose . . .	4-glucose-β-galactose	Animal sugar.]
Melibiose . . .	6-glucose-α-galactose	Trisaccharide raffinose.
Vicianose . . .	6-glucose-β-arabinose	Glycosides vicianin, gein, violutin.
Primeverose . .	6-glucose-β-xylose	Glycosides primeverin, gaultherin.
Rutinose . . .	glucose-rhamnose	Flavonols datiscin, rutin.
Rhamnoglucose . .	glucose-rhamnose	Anthocyanins (p. 210).
Strophanthobiose . .	glucose-cymarose	Saponin glycosides.

Sucrose

Sucrose, *Saccharose, Cane Sugar, Beet Sugar* is the substance commonly known as sugar. It occurs in such relatively large amounts in certain plants that it is an article of commerce; moreover, it is almost universally distributed in plants, especially in *green leaves* and *stems*, where it may either be a direct product of photosynthesis (p. 275) or a storage material. It occurs also in ripe sweet *fruits* such as pineapples, apples (5–6 per cent.), and strawberries (1 per cent.), in *roots*, *e.g.* of Beet, Carrot (7 per cent.), Radish, in the *sap* of the Sugar Maple (*Acer saccharatum*), and in some *seeds*. The *nectar* of flowers contains sucrose, and the bee hydrolyses this (by an enzyme secreted in the honey sac) to glucose and fructose, approximately equimolecular amounts of these hexoses being present in honey. In the stem of the Sugar-cane (*Saccharum officinarum*) sucrose occurs to the extent of 11–16 per cent., and by cultivation the percentage in the Sugar Beet (*Beta*) has been raised from 6 (A.D. 1747) to as much as 25.

Extraction. In the preparation of sucrose from the sugar-cane, the latter is pressed in mills and the resulting juice heated with lime, thus removing proteins by coagulation and acidic impurities by precipitation of the calcium salts. The filtrate, which contains a calcium derivative of sucrose, is treated with carbon dioxide to remove the calcium as carbonate. After filtration, the resulting sugar juice is concentrated by boiling under reduced pressure. *Raw sugar* crystallises out, and the residual syrup is called *molasses*.

Beets are extracted with water at 80° C., and the sugar extract treated as outlined above. In both purifications sulphur dioxide treatment is often used for decolorising the juices. At the refinery, the raw sugar, which is usually yellow or brown in colour, is dissolved in water, decolorised by filtration through animal charcoal, then re-evaporated and crystallised.

Crude molasses are used for making industrial alcohol by fermentation, and as binding materials in cattle-foods. Beet molasses contain a fair amount of nitrogen as protein, and also of potash; hence the residue from fermentation is often used in fertilisers.

Structure and Properties. Hydrolysis of sucrose, which is *dextro*-rotatory, $[\alpha]_D$ +66·5°, with dilute acid or with the enzyme **invertase** (occurring in yeast and in many green leaves), gives an equimolecular mixture of glucose, $[\alpha]_D$ +52·5°, and fructose, $[\alpha]_D$ −93°, hence the mixture has $[\alpha]_D$ −20°. Because of this change in the sign of rotation on hydrolysis, the mixture is called *invert sugar*. This hydrolysis of sucrose by invertase with the formation of invert sugar takes place in green leaves and in the ripening of some fruits, *e.g.* apples (p. 313). It has been shown that the fructose occurring in the sucrose molecule is not the normal or pyranose fructose isolated after hydrolysis but is γ-fructose or fructofuranose (p. 67). Sucrose is a **non-reducing** sugar, giving no reaction with ammoniacal silver nitrate or Fehling's solution, no colour on warming with alkali, and no osazone. The union of the two monosaccharides is therefore through both reducing groups. Sucrose only undergoes ordinary alcoholic fermentation if the yeast contains invertase as well as zymase; the latter alone has no effect. Sucrose can, however, undergo the lactic and butyric acid fermentations characteristic of lactose. It has been synthesised enzymatically.

EXPT. 22. *Sucrose*

1. Show that sucrose does not reduce Fehling's solution.

2. Show that on warming a sucrose solution with alkali no brown colour is developed.

3. Hydrolysis of sucrose with acid. To a sucrose solution in a test-

tube add a few drops of dilute sulphuric acid, and boil for two minutes. Cool, neutralise the acid with sodium hydroxide solution, and add Fehling's solution. A red precipitate of cuprous oxide is obtained on warming.

Lactose or *Milk Sugar* occurs in the milk of mammals but has not been detected in plants. The corresponding enzyme **lactase**, which hydrolyses lactose to glucose and galactose, does, however, occur in plants, having been found in Almonds and other seeds. Lactose is a *dextro*-rotatory reducing sugar, less sweet than sucrose.

Maltose or *Malt Sugar* occurs occasionally in the free state in plant tissues, *e.g.* in the Soya Bean (*Glycine hispida*). It is prepared by the action of the hydrolysing enzyme **diastase** on *starch*. Diastase is usually obtained from germinating Barley, but it is also present in many leaves and germinating seeds, where it regulates the balance between insoluble starch and the soluble diffusible sugar maltose, and it is probably concerned both in the synthesis and the hydrolysis of starch. *Ptyalin* of saliva is the same enzyme, hence the first change in the human consumption of starch is its hydrolysis to maltose. Maltose is a *dextro*-rotatory crystalline sugar, which reduces Fehling's solution, and gives a brown colour on being warmed with alkali. It is hydrolysed to two molecules of **glucose** both by dilute mineral acid and by the enzyme **maltase**, which is present in yeast and in many plant tissues. The intermediate production of maltose from starch in the manufacture of alcohol has already been described (p. 22). Since the maltose is present as a unit in the starch molecule, the mode of linking of the two glucose units is important; it has been shown that in maltose the α-modification of glucose is linked through its aldehydic hydroxyl to the hydroxyl in position 4 of the other molecule with the elimination of water (p. 74).

EXPT. 23. *Maltose*

1. Show that a solution of maltose reduces Fehling's solution on warming.
2. Warm a maltose solution with sodium hydroxide solution, and note the formation of a reddish-brown colour.
3. Prepare the osazone of maltose.

Cellobiose is obtained in the form of its acetyl derivative from *cellulose* by hydrolysis with sulphuric acid and acetic anhydride. It is built up of two **glucose** molecules linked as in maltose through the 1 : 4 positions, but here it is β-glucose that is present. It has been synthesised (Hudson). Cellobiose appears to stand in the same relation to cellulose as maltose does to starch, except that the yield of cellobiose from cellulose is only about 50 per cent., and therefore there still exists the possibility of other forms of linkage in the molecule of cellulose. An enzyme called **cellulase** has been discovered in many fungi, especially *Aspergillus*, *Penicillium*, and *Actinomyces*, which can effect this conversion of cellulose into cellobiose. The function of these organisms, which occur in great numbers in soils, is therefore of significance in the disintegration of plant residues and in soil fertility.

Trehalose also gives two glucose units on hydrolysis with acid. It is a *non-reducing* sugar, and therefore the glucose molecules are combined through the reducing groups. Trehalose occurs in Seaweeds, *e.g.* in the *Rhodophyceæ*, which contain about 10 per cent. of their dry weight of trehalose, and in some fungi, *e.g. Aspergillus niger*, mushrooms, and ergot. It has also been isolated from the Resurrection Plant (*Selaginella lepidophylla*) of the south-western states of America.

Gentiobiose is, like cellobiose, a glucose-β-glucoside, but the linkage is in position 6, and this has been substantiated by synthesis. Gentiobiose is the sugar present in the glycoside *amygdalin*, and it is also formed by partial hydrolysis of the trisaccharide *gentianose*.

TRISACCHARIDES

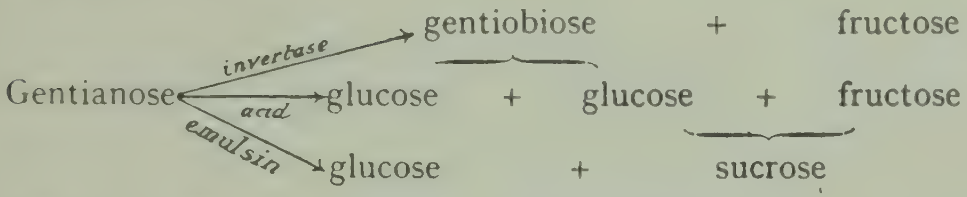
The trisaccharides are similarly constituted to the disaccharides, as they are built up from three monosaccharide molecules with the elimination of two molecules of water. This is shown by complete hydrolysis with dilute acid, but enzymatic hydrolysis can effect the decomposition in stages, the action of **invertase** and **emulsin** in particular giving clues to the nature of the linkage between the monosaccharide units.

$$\underset{\text{gentianose}}{C_{18}H_{32}O_{16}} + 2H_2O \longrightarrow \underset{\text{hexoses}}{3C_6H_{12}O_6}$$

The following trisaccharides are of importance in plants:—

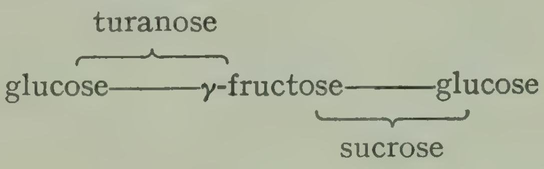
Gentianose .	glucose-glucose-γ-fructose	
Raffinose .	galactose-glucose-γ-fructose	
Melicitose .	glucose-γ-fructose-glucose	
Robinose .	galactose-rhamnose-rhamnose	Flavonol robinin
Rhamninose .	galactose-rhamnose-rhamnose	Flavonol xanthorhamnin

Gentianose is a *non-reducing* sugar occurring in the roots of the yellow Gentian (*Gentiana lutea*). Hydrolysis with acids gives two molecules of glucose and one of fructose; hydrolysis with emulsin gives sucrose and glucose, while with invertase, fructose and gentiobiose are obtained. The following scheme indicates these relationships:—



Raffinose is the most widely distributed trisaccharide. It occurs in the Sugar Beet, and in seeds of Cotton (*Gossypium*) and in Barley (*Hordeum*). It is also the main constituent of 'Eucalyptus manna', the exudation which develops on certain species of *Eucalyptus* as a result of insect punctures. It is a *non-reducing* sugar, and has exactly the same molecular structure as gentianose, except that the first glucose unit is replaced by galactose. Invertase hydrolyses the trisaccharide to melibiose and fructose, while emulsin gives sucrose and galactose.

Melicitose or **Melizitose** occurs in various exudations from trees; *e.g.* Briançon manna from Larch twigs (*Larix europæa*) and a manna from the Douglas Fir (*Pseudotsuga*). It can be partially hydrolysed to glucose and turanose, and also to glucose and sucrose. Its structure is therefore as follows:—



TETRASACCHARIDES

Stachyose is the only important tetrasaccharide in plants. It has been isolated from tubers of *Stachys tuberifera*, and occurs in the white Jasmine (*Jasminum officinale*), in the subterranean parts of the White Deadnettle (*Lamium album*), and in a manna from the Ash (*Fraxinus*). It is also probably identical with **lupeose,** which has been obtained from various leguminous seeds, including *Lupinus luteus*, *L. angustifolius*, and Peas (*Pisum*). Hydrolysis of stachyose with acid gives two molecules of galactose, one molecule of glucose, and one of fructose.

EXPT. 24. *Qualitative Tests for Sugars in Plant Tissues*

For non-green tissues such as roots and seeds, chop the material finely, place in a square of muslin, and squeeze in a small amount of distilled water in a beaker. Boil the extract and filter into an evaporating basin. If the filtrate is acid, neutralise with sodium hydroxide solution, and test a little of the liquid in a test-tube with Fehling's solution. If reduction takes place on warming, add Fehling's solution to all the liquid drop by drop until precipitation is complete. Filter off the copper oxide. Now hydrolyse the filtrate by boiling with dilute sulphuric acid for five minutes, neutralise with sodium hydroxide, and test with Fehling's solution. The first reduction shows the presence of *reducing sugars*, the second of *non-reducing sugars*. If no reducing sugars are present in the first instance, hydrolyse the extract and test as above for non-reducing sugars.

For green tissues, the extract must first be 'cleared'. Add to the original aqueous extract, drop by drop, a solution of basic lead acetate until no further precipitate is formed, then filter. This removes dissolved proteins, aromatic compounds, pigments, and glycosides. The filtrate is then treated with a dilute solution of sodium phosphate, added drop by drop, to remove lead from the solution. This is filtered again, and tested for reducing and non-reducing sugars as already described.

Similar extracts of either green or non-green tissues, when treated with phenylhydrazine hydrochloride as on p. 71, may yield the osazone of glucose and fructose.

EXPT. 25. *Extraction and Estimation of Total Sugars in Plant Tissue* (after Haynes and Archbold)

Apples are the simplest material to use. 30 grm. apples are cut finely and dropped into a wide-necked flask containing 100 c.c. boiling 95 per cent. alcohol to which is added 1 c.c. of concentrated ammonia (to neutralise acid). The alcohol is boiled on a water-bath for 15 minutes, then decanted through a filter-paper into a 500-c.c. distillation flask; another 100-c.c. portion of alcohol is added to the apple, boiled for 10 minutes, and decanted. This is repeated with a third 100-c.c. portion. The combined extracts are distilled under reduced pressure to remove the alcohol, and the residue diluted with distilled water, filtered if necessary, and made up to 250 c.c. (This must be tightly stoppered, preferably with a cork soaked in toluene to prevent mould growth.) The estimation is made volumetrically with Fehling's solution, using either methylene blue as internal indicator (preferable) or potassium ferrocyanide as external indicator. 34·639 grm. of $CuSO_4 \cdot 5H_2O$ is weighed accurately, dissolved in distilled water, and made up to 500 c.c. (Fehling's solution A). 173 grm. of Rochelle salt and 50 grm. of sodium hydroxide are dissolved in distilled water and made up to 500 c.c. (Fehling's B). A preliminary titration is made by placing 5 c.c. each of the two Fehling's solutions in a porcelain basin, and bringing to the boil over wire gauze. Some of the sugar solution diluted with three times its volume of water is run from a burette into the basin, the mixture being stirred with a glass rod, until the blue colour has disappeared. If the amount of sugar solution required is much greater or less than 10 c.c., another solution must be made up by suitably diluting some of the concentrated sugar solution so that about 10 c.c. is required for 10 c.c. of the mixed Fehling's solution.

With Methylene Blue (Lane and Eynon): A 200-c.c. flask is supported over a wire gauze, and 5 c.c. each of Fehling's solutions A and B introduced. A burette containing the diluted sugar solution is clamped so that the nozzle is just below the rim of the flask. The Fehling's solution is kept boiling gently, and the sugar solution run in slowly (10–15 minutes being taken for the titration), then about five drops of a 1 per cent. aqueous solution of methylene blue are introduced from a pipette just *before* reduction is complete. The discharge of the blue colour marks the end-point. Repeat the titration. 10 c.c. of Fehling's = 0·05 grm. of glucose or fructose. Calculate the percentage of **reducing sugar.**

With Ferrocyanide: The 10 c.c. of mixed Fehling's solution is placed in an evaporating basin, 40 c.c. of water added, the liquid boiled, and the sugar solution run in gradually from a burette. The Fehling's solution is gently boiled between each addition. Near the end-point, drops of the solution are removed on a glass rod to a tile, and a drop of potassium ferrocyanide solution, acidified with acetic acid, is added. A brown coloration indicates copper ferrocyanide and therefore unreduced copper in the solution. Continue the addition of sugar solution from the burette until the brown colour is no longer given.

Total Reducing Sugars after Hydrolysis. Measure out accurately 100 c.c. of the concentrated sugar solution into a flask, add 10 c.c. of concentrated HCl and heat on a boiling water-bath for 15 minutes to hydrolyse disaccharides. Neutralise the cooled solution with solid sodium carbonate, and make it up to 250 c.c. Estimate by Fehling's solution by either method, making an approximate titration first, then two accurate determinations. Find the total reducing sugars in 100 c.c. of the concentrated sugar solution, subtract the amount of reducing sugars present before hydrolysis in 100 c.c., and convert the remainder to per cent. **sucrose** in the original material. 1 grm. glucose = 342/360 sucrose = 0·95 grm. sucrose.

CHAPTER IX

STORAGE POLYSACCHARIDES. STARCHES

THE polysaccharides may be divided into two groups, storage and framework polysaccharides, according to their main function in the plant (p. 64). The starches comprise the first group, and occur either as the sole carbohydrate or with sugars as storage materials in seeds, tubers, roots, unripe fruits, and leaves. The general relationship of the polysaccharides to the monosaccharides has already been indicated, *viz.* they consist of large molecules built up from monosaccharide units with the elimination of water, which can be represented by the general formula $(C_6H_{10}O_5)_n$. They are hydrolysed to monosaccharides by dilute acid with greater difficulty than the disaccharides, and usually with the formation of intermediate products of less complexity belonging both to the polysaccharides and disaccharides. Specific enzymes are found in plant tissues which also effect this hydrolysis; in this way starch, which is temporarily stored in many green leaves, is hydrolysed and the soluble products translocated to storage organs such as tubers, seeds, or fruits, where the starch is again built up, the same enzymes probably effecting both hydrolysis and synthesis. During the germination of starch-storing seeds, or the sprouting of potato-tubers, or the ripening of fruits such as the banana, starch is again hydrolysed. Again, the conversion of starch into sugars is one of the mechanisms employed by plants in resisting cold and frost (p. 318), *e.g.* in the potato and in dormant twigs and buds of trees.

The polysaccharides are *amorphous* substances, and when soluble in water form only colloidal solutions; hence their isolation and purification are difficult. Their molecular weight cannot be determined accurately, but it is usually very large.

Starch

Occurrence. Starch, together with the sugars glucose, fructose, and sucrose, appears in the **chloroplasts** of the green leaves of many plants as a result of the photosynthetic process. Starch accumulates in the daytime when assimilation of carbon dioxide is taking place, and disappears at night when hydrolysis and translocation of the soluble products predominate. This is especially so in the dicotyledons, whereas many monocotyledons, and some dicotyledons belonging to the natural families *Compositæ*, *Umbelliferæ*, and *Geraniaceæ*, build up little or no starch in the leaves, but have high sugar concentrations instead. Starch grains are also deposited in the stroma of the **leucoplasts,** both in leaves and other parts of plants. It is the reserve material in many seeds, especially of the cereals (*Gramineæ*), and also of the *Leguminosæ*, where it occurs along with relatively large amounts of protein. Many roots of perennials, and tubers and corms contain starch, and in the growth of most fruits starch accumulates and is then hydrolysed during the ripening process. The cereal grains and potatoes are the main sources of starch for human consumption, *e.g.* maize contains about 70 per cent. starch, potatoes 20 per cent.; but other starches from tropical plants (*e.g.* arrowroot, sago, and tapioca starches) are also utilised. The occurrence of starch in the wood of trees, for instance, 5 per cent. or more in the summer wood of Birches, and its conversion into fat in winter has already been noted (p. 47).

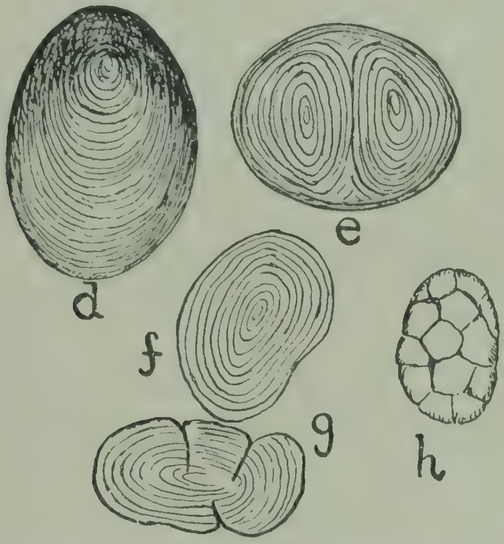


FIG. 4. Starch Granules (× 350): (*d*) and (*e*), potato starch; (*f*) and (*g*), pea starch; (*h*), rice starch (compound granule).

Starch always occurs in the form of *granules*. Under the microscope these are seen to possess a banded structure characteristic of the plant from which they are derived (fig. 4). The starch granules of many monocotyledons, *e.g.* Wheat, with their high sugar concentration, have a more uniform structure than those of dicotyledons, *e.g.* Potato, where a very marked banding occurs.

Preparation and Properties. Starch is manufactured principally from maize (U.S.A.), potatoes (Germany), and rice. The raw material is crushed with water and washed on to fine sieves. The starch, which is insoluble in cold water, is carried through the sieves mechanically with the water and separates out on standing.

The starch so obtained is a white amorphous powder. In hot water the granules burst, forming a bluish solution which is an emulsoid sol (p. 16); if this is concentrated it sets to a whitish gel on cooling. Alcohol and basic lead acetate also precipitate starch from such a solution. The most characteristic test for starch is the **blue** colour it gives with *iodine* solutions. Starch has no reducing properties; and is resistant to alkalis. When *heated*, starch first loses water, as it is hygroscopic, and then at about 200° C. it is transformed partly into **dextrins.** Boiling with dilute mineral *acids* hydrolyses starch *via* the intermediate products **dextrin,** $(C_6H_{10}O_5)_n$, and **maltose,** $C_{12}H_{22}O_{11}$, to **glucose,** $C_6H_{12}O_6$. This is not a clear-cut hydrolysis, as mixtures of all these products are obtained; on prolonged boiling, however, glucose is the final product. The enzymes **diastase** (or amylase) from germinating seeds, ptyalin of the saliva, and takadiastase from *Aspergillus oryzæ* also hydrolyse starch through dextrins to maltose; glucose is obtained only if the enzyme maltase is also present. Diastase is used in the manufacture of alcohol from starch (p. 22), while takadiastase, since it contains no maltase, is used in the estimation of starch in mixtures of carbohydrates.

$$\underset{\text{starch}}{(C_6H_{10}O_5)_n} \xrightarrow[\text{acid}]{\text{diastase}} \underset{\text{dextrins}}{(C_6H_{10}O_5)_n} \xrightarrow[\text{acid}]{\text{diastase}} \underset{\text{maltose}}{C_{12}H_{22}O_{11}} \xrightarrow[\text{acid}]{\text{maltase}} \underset{\text{glucose}}{C_6H_{12}O_6}$$

Expt. 26. *Examination of Starch*

Prepare a colloidal solution of starch as on p. 12.

1. Show that there is no reduction of Fehling's solution on boiling it with some of the starch solution.

2. Show that no colour is developed on heating some starch solution with sodium hydroxide solution.

3. Add one drop of iodine solution to a little starch solution in a test-tube, warm, and then cool the solution. Note that the original blue colour disappears on warming, but reappears on cooling.

4. Hydrolysis of starch with acid: Boil 75 c.c. of starch solution for 10 minutes with an equal volume of dilute sulphuric acid in a beaker with stirring. Test the solution with iodine from time to time, by removing a drop on to a tile on the end of a glass rod. Note that the blue starch colour changes first to a red colour then to the yellow of the iodine solution itself. At this stage, cool the solution, and neutralise with sodium hydroxide solution. Test some of the solution with Fehling's solution and show that it has reducing properties, and from the remainder of the neutralised solution try to isolate glucosazone (p. 71).

5. Hydrolysis of starch with diastase: Dilute 2 c.c. of the starch solution above with 20 c.c. of water, shake it up with a little diastase, and keep it in a warm place for an hour or so, testing the solution with

iodine solution on a tile from time to time as above. When no coloration is obtained, test the solution with Fehling's solution.

EXPT. 27. *Estimation of Starch in Potato*

Weigh out 3 grm. of potato chopped finely, and soak in 50 c.c. cold distilled water, with occasional stirring. The insoluble material is then filtered off and washed thoroughly with water. It is then transferred to a round-bottomed flask and heated for 2½ hours with 200 c.c. of water and 20 c.c. of concentrated hydrochloric acid on a water-bath. The liquid is then cooled, neutralised with sodium carbonate, filtered, and the filtrate transferred to a graduated flask and made up to 250 c.c. with water. The glucose in the solution is then estimated by Fehling's solution, using either methylene blue or ferrocyanide as indicator. Calculate the percentage of starch in the original material. (10 c.c. Fehling's = 0·05 grm. glucose, and 1 grm. glucose = 162/180 grm. starch = 0·9 grm. approx.)

[This method is only correct when no pentosans are present.]

Structure. Several forms of starch exist. Most starch granules contain at least two, *amylopectin* and *amylose*; the former composes the skeleton of the granule, and is less soluble and more resistant to hydrolysis than the amylose which it encloses. The two forms are separated from aqueous dispersions of starch by precipitation with butyl or amyl alcohol or with thymol. The paste-forming properties of starch are due to amylopectin. Amylose gives a blue colour with iodine, whereas amylopectin gives a red colour, which in the starch mixture is masked by the blue from the amylose portion. Most natural starches contain 20 to 25 per cent. of amylose.

Enzymatic hydrolysis of amylose by β-*amylase* (from ungerminated grains of Barley and Wheat; also from Soya Beans) results in complete conversion to maltose. Hence **amylose** is a **straight-chain polysaccharide,** consisting of chains of α-glucose units linked by primary valencies in the 1 : 4 position as in maltose. The formula for amylose is therefore correctly written as $C_6H_{11}O_6$–$(C_6H_{10}O_5)_n$–$C_6H_{11}O_5$. **Amylopectin,** on the other hand, is a **branched-chain polysaccharide** containing not only 1 : 4 linkages of α-glucose units, but cross-linkages through 1 : 6 positions between these polysaccharide chains (Freudenberg). Hydrolysis with β-amylase in this instance gives a little maltose and a residual dextrin of high molecular weight. Yeast contains an amylase mixture which can hydrolyse both 1 : 4 and 1 : 6 linkages, and the dextrin can be degraded still further. Saliva α-*amylase* can also do this. Hanes found that Potato juice and an extract from Peas contained an enzyme (1 : 4-*phosphorylase* or the P enzyme) which in the presence of phosphate could not only hydrolyse amylose but could effect the reverse *synthesis* to an amylose of about 80 glucose units. Natural amylose contains molecules of varying chain length, some being of this short type, but much of it contains up to 300 glucose units. Amyloses have been distinguished still further by their X-ray patterns. In the so-called 'A- and B-pattern' starches, the amylose is insoluble in water, and the X-ray diagram shows a linear extended chain of glucose units. In 'V-pattern' starch, or soluble starch, the amylose chain is coiled into a helix, alternate helices being laid down parallel to each other, but oriented in opposite directions.

Dextrins, *Starch Gum*, *British Gum*, are intermediate products obtained in the *hydrolysis* of *starch*; they occur temporarily in plants owing to the presence of diastase and starch. They can also be prepared by *heating* starch. Dextrins are white or yellowish powders, soluble in water to give gummy solutions which are *dextro*-rotatory. They are precipitated from aqueous solution by alcohol, but not by basic lead acetate (contrast starch). They possess no reducing properties when pure. They differ among themselves in the coloration given with *iodine* solution, **reddish-brown** and **blue** colours being obtained. As is indicated in the scheme on p. 84, dextrins are ultimately hydrolysed to **glucose** by the action of acids or of the appropriate enzymes.

Expt. 28. *Examination of Commercial Dextrin*

1. Dissolve 3 grm. of dextrin in water and precipitate it by adding twice its volume of alcohol (this removes any reducing sugars present). Filter off the precipitate, and use it for the following tests:—
2. Show that it does not reduce Fehling's solution.
3. Dissolve a little in water, add a few drops of dilute sulphuric acid and boil for 2 or 3 minutes. Cool, neutralise with sodium hydroxide solution, and show that if Fehling's solution is added, reduction takes place on warming.
4. Dissolve a little in water, add one drop of iodine solution, and warm. The original reddish-brown colour disappears on heating, and returns on cooling the solution.

Expt. 29. *Preparation of Dextrin from Starch*

Place 2 grm. of starch in an evaporating dish and heat cautiously on a sand-bath for 5 minutes, stirring constantly. Cool, add distilled water, and filter. Test portions of the filtrate with iodine, and with Fehling's solution, and show that alcohol gives a precipitate of the dextrin, but basic lead acetate does not.

Inulin

Occurrence. Inulin is a polysaccharide which occurs in the storage organs of plants of several natural orders, especially of the

Compositæ; it has been isolated from tubers of *Inula Helenium* up to 44 per cent. of their dry weight, from tubers of Dahlia (*Dahlia variabilis*) to 42 per cent., and of the Jerusalem artichoke (*Helianthus tuberosus*), and from roots of the Chicory (*Cichorium Intybus*) and Dandelion (*Taraxacum officinale*). Inulin is also present in many monocotyledons, either alone or with starch. Sometimes closely related species differ in the storage material; for instance *Scilla nutans* stores inulin but no starch, whereas *Scilla siberica* builds up both inulin and starch.

Structure and Properties. Inulin is built up mainly from γ-fructose (or fructofuranose) units in a similar manner to the assemblage of glucose units in the starch molecule, there being about thirty fructose units in the chain. Normal fructose (fructopyranose) is, however, obtained on hydrolysing inulin with acids or with the specific enzyme **inulase,** which occurs in the plant along with inulin. Small amounts of glucose and of a disaccharide of two molecules of fructose have, however, been obtained in constant amount in the hydrolysis of inulins from different plants, and therefore the molecule of inulin may be more complex than that of an anhydro-fructose. Inulin is a white, amorphous powder, dissolving in warm water to give a clear solution, from which it can be precipitated by alcohol or by basic lead acetate. It is non-reducing, and the brown colour of iodine solution is unaffected when added to it.

EXPT. 30. *Inulin*

1. Show that a solution of inulin does not reduce Fehling's solution.
2. Hydrolyse inulin by boiling a solution with a few drops of dilute sulphuric acid, and show that the resulting solution after neutralisation reduces Fehling's solution.
3. Show that inulin solution gives no colour change with iodine solution.

Lævans and Dextrans

Other polysaccharides composed of aggregates of fructose (lævulose) have been isolated from monocotyledons, especially from the grasses, and rhizomes of *Iris* species. They are grouped together as **lævans** or **lævulosans.** Individual names have been given to some; *e.g.* **phlein** from Timothy grass (*Phleum pratense*) and **irisin** from Iris. These lævans probably differ among each other and from inulin in the degree of polymerisation of the molecule.

Dextrans are similar polysaccharides built up of glucose (dextrose) units. They are water-soluble, resemble the dextrins, and are of smaller molecular size than starch. They have been shown to be present in the seedcoats of Rice (*Oryza sativa*) and of species of *Chelidonium*, in the epidermal cells of *Arum italicum*, in roots of Barley (*Hordeum vulgare*), and in the root-cap of the Onion (*Allium Cepa*). They are probably present as storage materials.

Glycogen or *Animal Starch* is the reserve polysaccharide characteristic of animal tissues, but it is also found in the seed of sweet Corn (*Zea Mays*), in some of the lower plant forms such as **fungi** (*e.g.* yeasts) and in some **algæ,** especially *Cyanophyceæ*. Glycogen is a white amorphous powder, soluble in water to give a colloidal solution. It is *dextro*-rotatory, and gives a red-brown coloration with iodine solution. It is non-reducing, and on hydrolysis with dilute acid gives glucose. It differs from starch in the size of the polysaccharide unit, as it contains fewer glucose residues.

CHAPTER X

FRAMEWORK POLYSACCHARIDES AND ASSOCIATED SUBSTANCES

THE skeletal structure of green plants is non-living, but is built up from inside the living cells, and differs morphologically and chemically with the age of the tissue. The main constituent of the cell-wall is **cellulose,** the most widely distributed of all naturally occurring organic compounds, though it is rarely the sole constituent. In most green plants, the fundamental framework of cellulose acquires increasing rigidity by successive incrustations in which physical association, adsorption, and chemical combination probably all play a part. The substances associated with cellulose may be grouped into four classes: (i) hemicelluloses, (ii) pectin, (iii) lignin, (iv) cutin and suberin. A broad distinction can be drawn between *lignified* and *unlignified* tissue: in the former type lignin plays its part in the skeletal structure, hemicelluloses are present in relatively large amounts, and there is little pectin; while the presence of pectin, small amounts of hemicelluloses, and no lignin characterise unlignified tissue.

The **celluloses** constitute at least half the plant cell-wall structure and occur in the form of *hollow fibres*, which, like the starch grains, are characteristic of the plant from which they are obtained. To the fibrous structure of cellulose is due its use in the textile industries (cotton, linen, and artificial silk) and in paper manufacture. The celluloses are condensation products of glucose, and are therefore polysaccharides. The seed hairs of the Cotton plant and the bast cells of the Hemp and Flax stalks are almost pure cellulose.

Hemicelluloses are condensation products of hexoses, usually other than glucose, *e.g.* mannose and galactose, with a pentose. In some cases they also contain uronic acid residues in the molecule and are therefore polyuronides. They are less resistant to hydrolytic agents than cellulose, and are universally distributed throughout the cell-walls of plants.

Pectin is a polyuronide, and occurs in the middle lamella of cell-walls of unlignified tissue.

In lignified tissues an amorphous substance called **lignin** is interpenetrated with cellulose fibres to form the cell-wall, while lignin is the sole component of the middle lamella of such tissues. Lignin appears to be an aromatic or hydroaromatic substance with a ketonic grouping, but it may be synthesised in the plant from carbohydrate (*e.g.* hemicellulose) when lignification takes place. *Wood* contains on an average 50–60 per cent. of cellulose, 25–30 per cent. of lignin, 4–12 per cent. of hemicelluloses, and small amounts of tannins, essential oils, etc., often grouped together as 'extractives'.

Cutin constitutes the cuticle of higher plants, while **suberin,** which is a cork-like material, may occur on the outer layers or in the inner cell-walls. They both consist chemically of oxidised and condensed fatty acids.

Gums and **Mucilages** are polyuronides and are therefore related chemically to the hemicelluloses and pectins. The mucilages are normal constituents of plants, but the gums are mostly produced under pathological conditions.

CELLULOSES

The cotton-wool cellulose has been the most completely investigated from a chemical standpoint, and is termed **normal** or **α-cellulose.** It is obtained in as much as 90 per cent. yield from the hairs attached to the seed of the Cotton plant (*Gossypium*). The stems of the Flax (*Linum usitatissimum*) and the Hemp plant (*Cannabis sativa*) yield about 70 per cent. of normal cellulose; linen, prepared from the former, is almost pure cellulose. Jute from species of *Corchorus*, and Esparto grass (*Stipa tenacissima*) consist of more complex celluloses, related to those in wood. The cellulose in the wood of conifers is more readily hydrolysed, and therefore probably simpler in molecular structure than that obtained from broad-leaved trees.

Properties of Normal Cellulose. Cellulose is insoluble in water and the ordinary organic solvents. It is chemically inert, as is evidenced by the process of filtration (filter papers are almost pure cellulose), and as would be expected from its structural function in plant life. Cellulose gelatinises and dissolves in *Schweizer's reagent* (a solution of cupric hydroxide in concentrated ammonia), and is reprecipitated as a gelatinous mass on acidification. This is used not only as a test for cellulose, but also in several technical processes in which a thin film of cellulose is required. Cellulose is also gelatinised and dissolved by concentrated solutions of *zinc chloride.*

The Action of Alkali. (i) Dilute sodium hydroxide solution has no effect on normal cellulose, and alternative treatment of fibre or wood pulp with chlorine and sodium hydroxide removes all incrusting substances including lignin, and is used for the isolation and estimation of cellulose. (ii) Concentrated sodium hydroxide causes the fibres to swell, and if kept stretched during the process, they become glossy. This effect, discovered by John Mercer, is used in the preparation of *mercerised* cotton. (iii) Cross and Bevan found that if carbon disulphide is added to cellulose pulp which has been treated with strong alkali, the cellulose loses its fibrous structure and becomes viscid owing to the formation of a complex derivative. This *viscose* can be spun into threads, and on decomposition with mineral acid, an artificial silk composed of a modified cellulose is obtained.

The Action of Acids. (i) If filter paper is immersed in 80 per cent. sulphuric acid and then washed rapidly in water, it becomes translucent and gives *parchment paper.* A partial hydrolysis has taken place, as the outer layers give a blue coloration with iodine, and the product is therefore called amyloid. (ii) Cold concentrated sulphuric acid dissolves cellulose: on dilution of this solution with water and boiling, **glucose** is obtained as the ultimate product. (iii) If, however, cellulose is heated with acetic anhydride and sulphuric acid, the acetyl derivative of the disaccharide **cellobiose** is obtained in about 50 per cent. yield. (iv) Dilute nitric acid converts cellulose into **oxycellulose**; this is acidic, and has reducing properties. It is probably a partially hydrolysed and oxidised product, a free aldehyde group having been oxidised to a carboxyl. Similar compounds termed **oxycelluloses** occur in the cell-walls of some plants, *e.g.* in the cereal straws. (v) Concentrated nitric acid forms ester-like compounds, the *cellulose nitrates*, in which the acid reacts with the hydroxyl groups in the cellulose molecule. A low nitrated cellulose called collodion cotton or pyroxylin, is used in solution as collodion; while completely nitrated cellulose is called gun-cotton and is used as a high explosive. (vi) Acetic acid also forms esters with cellulose; these *cellulose acetates* are used in the manufacture of one type of artificial silk (celanese), and also of aeroplane varnishes and cinematograph films.

Structure. Normal cellulose, in the form of its triacetate, can be hydrolysed quantitatively to glucose (Irvine). Cellobiose, which is 1 : 4-glucose-β-glucoside, may also be obtained by hydrolysis of cellulose. Hence cellulose appears to be built up from **β-glucose** units (contrast starch) joined through the 1 : 4-positions. In cellulose these units are linked together to give straight *chains* of different lengths, several of which are aligned parallel to each other, and held together by intermolecular forces, forming a micellar bundle or *micelle.* This is borne out by X-ray investigations (Hess, Sponsler and Dore, and Meyer and Mark). Several such micelles, with their long axes parallel, form the **unit fibre** of cellulose seen under the microscope; hence the morphological characteristics of cellulose are due to the ultimate arrangement of the glucose units in the molecule of cellulose. X-ray measurements of the actual size of these micelles have been made, and some have been found containing 100 to 200 chains; each chain consists of a large number of glucose units, the minimum being about 200. For raw cotton fibres, molecular weights as high as 480,000 have been obtained. The smallest X-ray unit, called the X-ray unit cell, is a straight chain containing 4, 6, and 8 glucose residues, depending on the source of the cellulose; this unit is repeated to form one of the long chains in the micelle.

β- and γ-Celluloses. These, like oxycellulose, occur in the cell-wall structure in varying amounts along with α- or normal cellulose. They differ from the latter mainly in being soluble in 17 per cent. sodium hydroxide solution, and in being less resistant to hydrolysis. They are separated from the alkaline solution by acetic acid, the β-form only being precipitated. They are composed of glucose residues condensed together, and probably differ from normal cellulose in the degree of complexity of the molecule.

EXPT. 31. *Normal Cellulose*

1. Place a little cotton-wool in Schweizer's reagent in an evaporating dish and rub it with a glass rod. Note that it becomes gelatinous and dissolves. Pour the liquid into a beaker full of dilute hydrochloric acid, when the cellulose will be precipitated.
2. Place some cotton-wool in an evaporating dish with a little concentrated sulphuric acid. When the cellulose has all dissolved, pour the solution into about four times its volume of water in a conical flask and boil for 15 minutes. Cool, neutralise with sodium hydroxide solution, and show that it reduces Fehling's solution.
3. Add some concentrated sulphuric acid to half its volume of water in a test-tube, and cool under the tap. Place the acid in an evaporating dish, immerse a piece of filter-paper in the liquid, then transfer it immediately to a beaker full of water. Wash the paper well, and pin it up to dry. Examine the paper, note its toughness, and show that a drop of iodine solution on it gives a blue colour.

HEMICELLULOSES

Occurrence and Function. The hemicelluloses form part of the *cell-wall structure* of plants, especially in lignified tissues. They are separated from cellulose by extraction from plant tissue with four per cent. sodium hydroxide solution, and can be reprecipitated by acetic acid in the presence of alcohol. The term hemicellulose is a loose one, covering at least two different groups of polysaccharides; *viz.* (*a*) the **cellulosans,** which are short-chain polysaccharides associated and oriented with the cellulose micelles; these give *sugars* on acid hydrolysis; (*b*) the **polyuronide hemicelluloses,** which are amorphous polysaccharides encrusting the cellulose fibres, and may in part be chemically linked to lignin. On acid hydrolysis they give *sugars* and small amounts of *uronic acids*. Hemicelluloses, which appear to function solely as structural material, are present in the *wood* and *leaves* of many trees, such as Lime, Chestnut, Apple, Beech, Oak, and the Conifers, and in some *fruits*, *e.g.* the Apple. Hemicelluloses also function as reserve food materials; they have been called **reserve celluloses,** and are mostly of the cellulosan type. They occur in the cell-walls of the *endosperm* of *seeds*, especially those which are comparatively poor in starch or oil. They have been isolated from the endosperm of seeds of some Palms (*Phytelephas* and *Cœlococcus*), the product from the former being called 'vegetable ivory', and also from seeds of Dates (*Phœnix*), Coffee (*Coffea arabica*), Lupin (*Lupinus*), Nasturtium (*Tropæolum*), Pea (*Pisum*), and Beans (both *Vicia Faba* and *Phaseolus vulgaris*).

Structure. The hemicelluloses differ from the true celluloses in that they can be hydrolysed by boiling with dilute mineral acids. The products are sugars, usually a mixture of *hexoses*, especially glucose, galactose, and mannose, and of *pentoses*, especially arabinose and xylose. The hemicelluloses of wood and many non-lignified tissues, *e.g.* Flax, Wheat bran, Maize cobs, have also been shown to contain small amounts of the **uronic acids,** galacturonic and glucuronic acids (p. 69). These acids are very easily decomposed by dilute mineral acid with the loss of carbon dioxide, and hence they may be present in small amounts in the molecules of many hemicelluloses in which they have not yet been recognised.

The wood hemicelluloses have a higher uronic acid content than those from unlignified tissue, and appear to be considerably more complex; hemicelluloses of younger, less lignified tissue, *e.g.* Oat hulls, Wheat straw, yield mainly arabinose and galactose. Lignified tissue on the other hand yields xylose, glucose, and mannose. There is, however, a difference between the hemicelluloses of conifers and of deciduous trees, as shown by their hydrolysis products; hemicelluloses from Pine wood give about 25 per cent. xylose, 21 per cent. glucose, and 43 per cent. mannose, while Beechwood hemicellulose yields 74 per cent. xylose, 20 per cent. glucose, and only 3 per cent. mannose. Sapwood and heartwood from the same species of Oak contain different hemicelluloses. Hydrolysis products from both consist of xylose and a methylated uronic acid, but sapwood also yields glucose, which is absent when the sapwood matures to heartwood.

The cellulosans give mainly one or two sugars on hydrolysis; those which give pentoses are grouped together under the name **pentosans;** similarly those yielding principally hexoses, which may be called *hexosans*, are designated **mannans, galactans, galactomannans,** etc. That many of the hemicelluloses in seeds function as reserve materials is shown by their disappearance during germination, with the formation of **sugars.** An enzyme **cytase,** which effects this hydrolysis, is present in such seeds, and has been isolated from germinating Lupin seeds and the stones of Dates. Cytase is very closely related to cellulase.

Pentosans. The formation of pentosans in plants is favoured by dry conditions and high temperatures. Many *succulents, e.g.* Cacti, contain considerable amounts of pentosans. It has been suggested that owing to their absorption of water, the pentosans *increase the hydration capacity* and therefore assist in retaining water in plant tissues which have to withstand drought. There is, however, no rigid proof of this. As no enzyme has been found which hydrolyses pentosans it is doubtful if they ever act as reserve materials, although they are found in seeds. Like the pentoses, the pentosans break down with concentrated hydrochloric acid to give *furfural* (p. 72). They are also distinguished by giving insoluble *copper derivatives* on treatment with Fehling's solution in the presence of excess alkali; the pentosan can be regenerated from such derivatives by dilute acid. The two most widely distributed pentosans are **xylan** and **araban,** which give xylose and arabinose respectively on hydrolysis. Xylan occurs in lignified cell-walls, especially in the wood of deciduous trees, and is the chief constituent of wood gum. It also occurs in many of the cereal straws and 'brans', in Esparto grass, and Bamboo, and in the pods of Beans and Peas. Wheat bran and straw each contain about 25 per cent. of pentosans, while Maize bran contains about 40 per cent. Araban is associated with xylan in woods, and also occurs in several gums.

EXPT. 32. *Detection of Pentosans in Sawdust or Bran*

Boil a small amount of the material in a conical flask on a water-bath several times with rectified spirit. Filter, or decant off the alcohol each time, to remove all sugars and glycosides present. Evaporate off all traces of the alcohol from the material in the flask, then make the following tests:—

1. Warm some of the material with concentrated hydrochloric acid,

and place aniline acetate paper in the mouth of the test-tube. A pink or red colour is developed.

2. Warm some of the material with concentrated hydrochloric acid, then add solid phloroglucinol; a red colour is obtained.

Hexosans. **Mannans,** mixed with polysaccharides built up from mannose with other hexoses and pentoses, are widely distributed in plants, especially in seeds of many members of the *Leguminosæ*, *Umbelliferæ*, and *Coniferæ*. *Vegetable ivory*, the endosperm of the Palm, *Phytelephas macrocarpa*, consists largely of mannan, and is the principal source of mannose. Mannans can also be isolated from many of the plant mucilages, *e.g.* Salep mucilage from tubers of the *Orchidaceæ*, while wood contains about 0·5 per cent. of mannan. **Galactans,** which give galactose on hydrolysis, also occur along with more complex polysaccharides containing galactose, as reserve hemicelluloses in the endosperm of many seeds, including those from several of the *Leguminosæ*. *Arabogalactans* have been isolated from the wood of certain conifers, especially the Larch. Hydrolysis gives the pentose arabinose and the hexose galactose in the proportion of 1 : 6.

Lichenin $(C_6H_{10}O_5)_n$, is a starch-like material which gives **glucose** on acid hydrolysis. It occurs in Iceland Moss (*Cetraria islandica*), but unlike starch it is uniformly distributed among the cells, and is not deposited in granules. It is very closely related to cellulose, and may be classed as a *reserve cellulose* or a hexosan. An enzyme **lichenase** also occurs in plants which will hydrolyse lichenin to **cellobiose,** but if the enzyme cellobiase is present as well, glucose is the product. Associated with lichenin is *iso***lichenin** which gives a blue colour with iodine, and is probably a starch.

PECTIN

Pectin belongs to the acid polysaccharide or **polyuronide** class of carbohydrates, and is characteristic of *unlignified tissues*, where it is closely associated with both cellulose and hemicelluloses. It occurs both in the *cell-wall* and in the *middle lamella* of **unripe fruits** such as Apples, of **fleshy roots** such as Turnip, Carrot, and Beet, in the rinds of Citrus fruit, and in pods of Peas and Beans. From these tissues it is extracted with boiling water or ammonium oxalate solution. Pectin probably acts as a cementing agent in holding the cellulose fibres together, but a chemical union between groups in pectin and cellulose molecules has not been entirely disproved. Pectin occurs in a water-soluble form in **ripe fruits** such as Red and Black Currants, Gooseberries, and Oranges, and in some **roots,** *e.g.* Carrot, Turnip, and Beet.

Structure. Ordinary preparations of pectin contain cellulosans, and earlier results on hydrolytic fission gave erroneous results because of the presence of cellulosan degradation products, *viz.* arabinose and galactose. Hydrolysis of pure preparations of pectin give **galacturonic acid,** and the simplest substance, **pectic acid,** consists of a chain of galacturonic units linked in the 1 : 4 positions, similar to the glucose chain in the starch molecule. In *Citrus* pectin the minimum length of the chain is 8 to 10 galacturonic units. In the plant, these chains are methylated to varying amounts. Acid or alkaline hydrolysis, therefore, also furnishes **methyl alcohol,** and hydrolysis with lime water leads to an insoluble calcium pectate. An enzyme **pectase** occurs in several fruits, roots, and some leaves, *e.g.* Clover, and demethylates pectin to pectic acid. This change occurs in the spontaneous setting of some fruit juices, but the making of jams and jellies involves a *physical* change, and is a *precipitation* of *pectin* as a gel in the presence of *sugar* and *acid.*

EXPT. 33. *Pectin*

Take a chopped ripe apple or a handful of red or black currants or gooseberries and squeeze through muslin into a little water in a beaker. Precipitate the *pectin* by adding at least twice the volume of alcohol. Allow the gelatinous mass to stand for a little, then filter it off and wash with alcohol. Dissolve it in the minimum amount of water and perform the following tests:—

1. Show that pectin gives no precipitate with hydrochloric acid.
2. Show that it gives no precipitate with calcium chloride.
3. Show that it gives a gelatinous precipitate with lime water, due to hydrolysis of the pectin and formation of *calcium pectate.*
4. Hydrolyse some of the pectin by making the solution alkaline with sodium hydroxide solution, and allowing to stand for 10 minutes or so. Show that portions of this solution give gelatinous precipitates of (*a*) *pectic acid* on acidification with concentrated hydrochloric acid, and (*b*) *calcium pectate* on addition of calcium chloride solution.
5. Make an extract of pectase by pounding some clover leaves or young carrot root with a little water in a mortar and filtering. Add the extract to some of the pectin solution, when a gelatinous precipitate of *pectic acid* is obtained.

GUMS AND MUCILAGES

The gums and mucilages resemble the pectins and the wood hemicelluloses in being **polyuronides.** The gums are in most cases *abnormal products* in the plant due to *pathological conditions* brought about either by unfavourable conditions of growth or by injury; they may therefore be produced from the normal cell-wall constituents rather than synthesised *de novo.* The mucilages, on the other hand, are constant components of certain plant tissues, and are probably built up in a parallel manner to the hemicelluloses.

Gums. The gums are mainly derived from *trees*, and are produced in some cases by abnormal growth conditions, *e.g.* gum tragacanth, in others as a result of injury including that due to bacterial or fungal invasion, *e.g.* wound gum. Most of the gums are soluble in water, giving sticky colloidal solutions from which they may be precipitated by alcohol as translucent, amorphous solids. The gums are all *lævo*-rotatory, and can therefore be distinguished from dextrin. Hydrolysis of gums occurs only after prolonged boiling with acids, and yields mixtures of sugars, usually pentoses and hexoses, and uronic acid; but the percentage composition of any particular gum is not always constant. Hydrolysis rarely results in the formation of galacturonic or glucuronic acids themselves; it yields rather uronic acid residues still combined with sugars, *e.g.* galactoglucuronic acid. The general term **aldobionic acid** is sometimes used for such substances. These units may be resistant to further hydrolysis owing to the formation of a lactone (p. 70) by the elimination of water between the carboxyl group and a hydroxyl group. This aldobionic structure of the acids from the gums was not at first recognised, and hence names such as *arabic acid* were given, which merely denoted the source of the various acids (*e.g.* gum arabic). The gums usually exhibit the acid character of the uronic acid part, and are present in nature as **salts** of calcium, magnesium, and potassium.

The following are some of the gums that are isolated in quantity, and have commercial value:—

Gum Arabic is obtained as an exudation from the branches of species of Acacia, especially *Acacia senegal*, native to the Sudan, but originally shipped *via* Arabian ports, hence the name. It occurs as a mixture of metallic salts, and on hydrolysis yields galactose, arabinose, probably also rhamnose, and galactoglucuronic acid.

Gum Tragacanth is produced by several species of *Astragalus* (of the *Leguminosæ*) in Turkey and Persia. It is produced by the metamorphosis of the medullary rays under unfavourable conditions of growth, but may also be obtained by wounding the stems. On hydrolysis it yields arabinose, galactose, xylose, and an aldobionic acid.

Wound Gum is formed in the wounding (sometimes by bacterial action) of trees, especially of Peach and Cherry. Since these gums swell on the absorption of water, they sometimes block the lumens of conducting tissues and cause wilting. When wound gum is produced in cells surrounding surface wounds, these are staunched.

Cherry Gum, from *Prunus Cerasus* and *P. Padus*, and the chemically similar **Plum** (from *P. domestica*) and **Almond** (from *P. Amygdalus*) **gums,** all known commercially as **cerasin,** occur in the stems and branches of these and other species of *Prunus*, and exude from cuts in the bark. On hydrolysis a mixture of sugars is obtained, principally arabinose, and an aldobionic acid.

EXPT. 34. *Gum Arabic*

Add a little water to some gum arabic in an evaporating dish, warm gently, and stir. A thick, sticky solution is obtained which does not gel on cooling. Make the following tests on the solution:—

1. Show that alcohol precipitates the gum.
2. Show that no coloration is given with iodine solution.
3. Show that Fehling's solution is not reduced.
4. Boil some of the solution with concentrated hydrochloric acid, and test the vapour with aniline acetate paper. The pink colour due to pentoses is developed.
5. Heat the solution with concentrated hydrochloric acid and a little phloroglucinol. The red colour due to pentoses is produced.
6. Hydrolysis of gum arabic: To about 20 c.c. gum arabic solution in a beaker add twice its volume of dilute sulphuric acid and boil for 10 minutes. Cool the solution and divide into two portions. To one add concentrated hydrochloric acid and phloroglucinol and boil; a red colour shows the presence of pentoses. Neutralise the other portion with sodium hydroxide solution, and show that it now reduces Fehling's solution.

Mucilages. The mucilages are normal constituents of certain plants. They are present as a semi-solid mass dispersed throughout the cell, and may occur in any organ of the plant. Some plants possess mucilage-secreting hairs, others special mucilage cells or sacs, while in others the mucilage is concentrated in the outer walls. Mucilages absorb water readily and swell, and increase the hydration capacity of the plant. They are found in **xerophytes,** *e.g.* in many of the *Catacеæ*; they are also present in young **buds**, *e.g.* Poplar, and in some **aquatic plants** in which they prevent too rapid diffusion of water through the cell-wall. Mucilages also occur in **seeds,** *e.g.* Flax, where they occur on the surface, and Mustard (*Brassica alba*), which contains special cells in the seed-coat; in these cases they may possibly assist in the imbibition of water necessary for germination. Other plant mucilages besides those mentioned above occur in tubers of the *Orchidaceæ*, where they may possibly act as a reserve food material, also in bulbs of *Scilla*, *Allium*, and Tulip (*Tulipa*), roots and flowers of Hollyhock (*Althæa rosea*), and fruits of Mistletoe (*Viscum album*). Mucilages form colloidal solutions in water; these are slimy, and the mucilage can be precipitated from them by alcohol.

Linseed mucilage has been the most studied from a chemical point of view; hydrolysis with acid gives a mixture of *sugars* including glucose, galactose, arabinose, and xylose, and *aldobionic acids*. A rhamnogalacturonic acid has been isolated from linseed mucilage.

Agar-agar is a mucilage from Seaweeds (*Rhodophyceæ*) and appears to contain sulphuric acid in the molecule, probably as an ester.

EXPT. 35. *Mucilages*

Warm a little agar-agar (or linseed mucilage) with water. A thick, slimy solution is obtained. Make the following tests on this solution:—

1. Show that alcohol precipitates the mucilage.
2. Show that no colour is given with iodine solution.
3. Show that Fehling's solution is not reduced.
4. Boil some of the solution with concentrated hydrochloric acid, and show with aniline acetate paper that pentoses are produced.
5. Heat the solution with concentrated hydrochloric acid and a little phloroglucinol, when the red colour due to pentoses will be produced.
6. Boil about 20 c.c. of the solution with twice its volume of dilute sulphuric acid in a beaker for 10 minutes. Cool the solution, and show that after being neutralised with sodium hydroxide solution it reduces Fehling's solution.

LIGNIN

Lignin is the main constituent of lignified tissues next to cellulose. It forms from 25–30 per cent. of the wood of trees, the conifers having the higher content, from 15–20 per cent. of straws, and from 30–40 per cent. of Heather. It can be readily detected in the plant by several colour tests.

EXPT. 36. *Lignified Tissues*

1. Dissolve a little aniline in excess dilute hydrochloric acid, and soak some wood shavings, straw, or a match stick in the solution. A bright yellow colour is developed, due to the presence of lignin.
2. Dip wood shavings or similar lignified tissue in an alcoholic solution of phloroglucinol, then dip in concentrated hydrochloric acid. A magenta-red coloration is obtained.

In the manufacture of paper from wood, the lignin is dissolved out by cooking the pulp with sulphur dioxide and lime water, when soluble sulphonic acid derivatives of lignin are formed. In the estimation of cellulose, the removal of lignin is effected by the chlorine and caustic soda method (p. 90). Lignin can be precipitated from these alkaline solutions giving various 'precipitated lignins' and 'lignic acid.' On the other hand, the cellulose and hemicellulose can be removed from the tissue by hydrolysis in the cold with concentrated hydrochloric acid (Willstätter) leaving lignin. It is probable (Freudenberg) that there is no chemical combination of cellulose and lignin in the cell-wall, but that **secondary lignin,** which is amorphous, of high molecular weight, and highly polymerised, forms the middle lamella of lignified tissue; in addition, the primary and secondary layers round the lumen in soft woods are held to be composed of lignin interpenetrated and packed by cellulose micelles, which form a regular pattern round the fibre axis. This lignin-cellulose framework may further be interpenetrated by the hemicelluloses and the small amounts of pectin in lignified tissue.

Structure. Lignin contains hydroxyl groups, methoxyl groups, methylene groups (splitting off formaldehyde on hydrolysis), and has also an aldehydic or a ketonic group. The methyl alcohol in 'pyroligneous acid', for instance, is derived from the lignin of the wood. Fusion of lignin with potassium hydroxide yields *protocatechuic acid* (p. 183) and *catechol* (p. 176), and boiling with strong hydrochloric acid yields *furfural* (p. 72). Oxidation of lignin from Gymnosperms (*e.g.* Spruce) gives rise to *vanillin* (p. 183), whereas *syringaldehyde* (p. 184) in addition to vanillin is obtained from Angiosperms (*e.g.* Maple, Aspen, Bamboo). Again, *coniferyl alcohol* (p. 183) is present as the glucoside coniferin (p. 111) in all young plant tissues of conifers and in the cambial sap of the Spruce. Lignins are therefore polymers of a fundamental aromatic unit related to these compounds. The number of methoxyl groups and the degree of polymerisation probably vary with the type of plant, the age of the plant, and conditions of growth. Ultraviolet adsorption spectra measurements and X-ray analysis confirm this polymer hypothesis. Further discussion of the possible structure is included in the aromatic section (*loc. cit.*).

CUTIN AND SUBERIN

Cutin. Cutin forms the **cuticle** or superficial water-impermeable deposit on the epidermis of the leaves and stems of higher plants. Only a thin cutin layer is formed by plants that are shade-loving or grow in damp places, while desert plants have usually a thick cuticle. The amount of cuticle depends also on the amount of fat elaborated by the plant, *e.g.* heath plants always contain large amounts of fat and have a thick cuticle. There is no cellulose in the cuticle itself, although in some cases in the adjacent lamellæ the primary wall of cellulose is impregnated with deposits of cutin, forming the so-called cutinised layers. Cutin is resistant to cellulose solvents, such as zinc chloride solution and Schweizer's reagent, and as the cuticle remains unaltered by these solvents, there is no justification for the term cuto-cellulose. On the other hand, the cuticle can be removed from the cellulose in the primary wall by boiling with aqueous potassium hydroxide—that is, by *saponification*—or by *oxidation* with hypochlorite (*eau de Javelle*). Cutin is not soluble in the fat-solvents, therefore is more complex than the lipoids. Cutin is, in fact, not one chemical substance but a complex mixture consisting mainly of *unsaturated fatty acids* which have undergone *condensation* and *oxidation*, and of soaps and esters of such complexes. Priestley has explained cutin formation by the movement of unsaturated acids, probably as the soluble soaps with sodium and potassium, from meristematic tissue, on differentiation, to the surface of the cell-wall, where, in contact with air, and probably accelerated by light, they undergo oxidation and condensation, so that a varnish-like skin is deposited as in the case of a drying oil. It has been shown that where sodium and potassium ions are present in large amounts in the soil and the cell-sap, a thicker cutin layer is obtained than when calcium predominates, the greater solubility of the sodium and potassium soaps enabling them to move more freely and to accumulate on the surface.

Suberin. Suberin is the cork-like material which occurs in the median lamella of the walls of **periderm** cells lying between the outer middle lamella and the inner cellulose layer. Commercial *cork* comes from the periderm of the Cork Oak (*Quercus suber*). Suberin, like cutin, is a mixture of complex substances derived by *condensation* and *oxidation* from *fatty acids*. Hydrolysis of suberin with alcoholic sodium hydroxide gives a mixture of sodium salts of acids, from which two crystalline acids, phellonic, $C_{22}H_{43}O_3$, and phloionic, $C_{22}H_{40}O_7$, have been obtained, together with an amorphous acid, suberinic acid, $C_{17}H_{30}O_3$. These show the properties of fatty acids, being soluble in the fat-solvents, but if they are heated they give **anhydrides** which are no longer soluble in these solvents. Suberin itself probably contains derivatives of this anhydride-like nature. The formation of a suberised layer on the cut surface of a Potato tuber exposed to the air is an instance of the conversion of carbohydrate into fat in the plant. After the surface is cut, starch disappears, there is an abnormal production of sugars, and the walls of the cells become suberised, presumably through the intermediate formation of fatty acids from the sugar.

CHAPTER XI

GLYCOSIDES

The glycosides (p. 64) are very widely distributed in plants, especially in roots, bark, and fruits, and to a less extent in leaves.

Structure. On acid *hydrolysis*, glycosides yield a **sugar** and one or more other products which are relatively reactive substances and for which the general term **aglucone** has been suggested. The derived glycosides are usually more stable, and more soluble, especially in water, and therefore in the cell-sap, than their aglucones. In the plant an *enzyme* is often secreted in the same tissue, but in a different cell from the glycoside. Rupture of the cell-walls by injury, and also during the germination process in seeds, brings the enzyme into contact with its substrate. The sugar most commonly present is **glucose,** which is combined through the aldehydic hydroxyl group with a hydroxyl group in the aglucone with the loss of a molecule of water. Two types of glucosides are therefore possible, corresponding to the two methyl-glucosides (see formula (III), p. 66); most of the naturally occurring glucosides are β-glucosides. The distinction between the two types is provided by the enzymes *maltase* and *emulsin*, the former hydrolysing the α-glucoside, the latter the β-glucoside linkage. As sugars other than glucose also occur in these compounds, the general name **glycoside** has been suggested for the whole class (Armstrong). Disaccharides are also present in the naturally occurring glycosides; they are listed on p. 75, and it will be seen that several of them are peculiar to one or a few glycosides. The additional sugar residue in the glycoside always increases its solubility. In several of the anthocyanin pigments, which are glycosides, two separate sugar residues are present, combined with two hydroxyl groups in different positions in the aglucone molecule.

Isolation and Properties. The glycosides are white crystalline substances when pure, and usually have a bitter taste. They are soluble in water and in most of the common organic solvents except ether. Where a corresponding enzyme is present in the same tissue, steps must be taken in the isolation to prevent the enzyme from coming in contact with the substrate: the usual method employed is therefore to drop the tissue into boiling water or boiling alcohol, which inactivates the enzyme.

Classification. The glycosides are usually classified according to the nature of the aglucones. The following groups will be discussed in turn: (I) the Cyanophoric glycosides, (II) the Mustard Oil glycosides, (III) the Saponins, (IV) the Phenolic, Coumarin, and Hydroxyanthroquinone glycosides. Many of the anthoxanthin and all the anthocyanin pigments are glycosides (Chap. XIX), while probably all the tannins are also glycosides (p. 187).

I. CYANOPHORIC GLYCOSIDES

The cyanophoric glycosides are characterised by yielding **hydrocyanic acid** (prussic acid), HCN, on hydrolysis with either mineral acid or the appropriate enzyme. They are widely distributed in plants, hydrocyanic acid having been found in 148 species of 41 families investigated. The simplest test for hydrocyanic acid is the red coloration given with sodium picrate. When the specific enzyme occurs in the same tissue, bruising the material, or, more quickly, autolysing it with chloroform, is sufficient to effect the hydrolysis, and the hydrocyanic acid may be detected both by its odour (of almonds) and by the above colour reaction.

EXPT. 37. *Cyanophoric Glycosides*

Dip strips of filter-paper in a saturated solution of picric acid, and dry by suspending in air. When these strips are moistened with sodium carbonate solution and inserted with the cork in a flask or test-tube, they will show the presence of hydrocyanic acid by changing colour to orange and finally to a brick red. Show that leaves of the Cherry Laurel, Bird Cherry, Elder, or Hawthorn give off hydrocyanic acid when torn into small pieces or autolysed with a few drops of chloroform and placed in stoppered test-tubes containing sodium picrate paper.

Break up one sweet almond and one bitter almond and place in separate test-tubes; only the bitter almond contains both glycoside and enzyme, and therefore gives off hydrocyanic acid; shown by the coloration of sodium picrate paper.

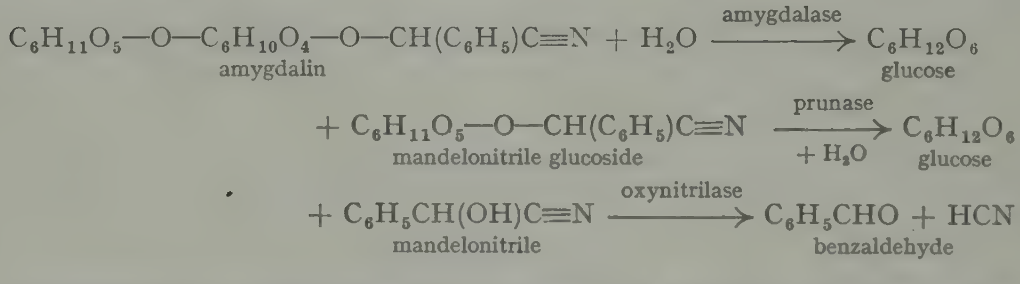
The following table shows the composition and distribution of the most common cyanophoric glycosides:—

Glycoside	*Source*	*Hydrolysis Products*
Prunasin	*Prunus*	Glucose + benzaldehyde + HCN
Prulaurasin	*Prunus*	Glucose + benzaldehyde + HCN
Sambunigrin	*Sambucus*	Glucose + benzaldehyde + HCN
Amygdalin	*Prunus, Pyrus*	Gentiobiose + benzaldehyde + HCN
Vicianin	*Vicia*	Vicianose + benzaldehyde + HCN
Dhurrin	*Sorghum*	Glucose + *p*-hydroxybenzaldehyde + HCN
Phaseolunatin	*Phaseolus, Linum*	Glucose + acetone + HCN
Lotusin	*Lotus*	Gentiobiose + lotoflavin + HCN

Of the many common British plants, besides those listed above, which contain cyanophoric glycosides are leaves of the Black and Red Currants (*Ribes nigrum, R. rubrum*), of the Gooseberry (*R.*

Grossularia), of the Vetches (*Vicia sativa, V. hirsuta*), and of the Hawthorn (*Cratægus Oxyacantha*).

Amygdalin, $C_{20}H_{27}O_{11}N$, is the most important member of this group. It occurs in the seeds of the bitter Almond but is absent from the sweet or cultivated Almond (both *Prunus Amygdalus*). It also occurs in kernels of the Plum (*Prunus domestica*), Peach (*P. Persica*), Apricot (*P. Armeniaca*), and Cherry Laurel (*P. Laurocerasus*), in the latter up to as much as 4 per cent., and in other members of the *Rosaceæ*, including seeds of the Apple (*Pyrus Malus*) and of the Mountain Ash (*Pyrus Aucuparia*). The glycoside is obtained by expressing the oil from the seeds and extracting the residue with hot alcohol. Complete hydrolysis with dilute acid gives two molecules of **glucose,** one molecule of **benzaldehyde,** and one of **hydrocyanic acid.** An enzyme-complex called 'emulsin,' which also hydrolyses amygdalin, is present in both bitter and sweet Almonds; this hydrolysis takes place in several stages, and it has been shown that at least two, and probably three, enzymes are present in emulsin. The first, **amygdalase,** hydrolyses amygdalin to one molecule of glucose and one of a *dextro*-rotatory mandelonitrile glucoside; the second, **prunase,** hydrolyses mandelonitrile glucoside to glucose, benzaldehyde, and hydrocyanic acid; this latter reaction may occur in two stages, as the hydrolysis by prunase may give mandelonitrile, leaving a third enzyme, **oxynitrilase,** to decompose the mandelonitrile to benzaldehyde (p. 180):



$$\underset{\text{amygdalin}}{C_6H_{11}O_5{-}O{-}C_6H_{10}O_4{-}O{-}CH(C_6H_5)C{\equiv}N} + H_2O \xrightarrow{\text{amygdalase}} \underset{\text{glucose}}{C_6H_{12}O_6}$$

$$+ \underset{\text{mandelonitrile glucoside}}{C_6H_{11}O_5{-}O{-}CH(C_6H_5)C{\equiv}N} \xrightarrow[+\,H_2O]{\text{prunase}} \underset{\text{glucose}}{C_6H_{12}O_6}$$

$$+ \underset{\text{mandelonitrile}}{C_6H_5CH(OH)C{\equiv}N} \xrightarrow{\text{oxynitrilase}} \underset{\text{benzaldehyde}}{C_6H_5CHO} + HCN$$

Amygdalase is also present in *yeast,* as extracts of the latter hydrolyse amygdalin to mandelonitrile glucoside.

EXPT. 38. *Isolation of Amygdalin from Bitter Almonds*

Pour boiling water over a dozen bitter almonds, remove the testas, then chop up the almonds and boil them in a flask with ethyl alcohol on a water-bath. Filter off the alcoholic extract and evaporate it to dryness on the water-bath. Extract the amygdalin from the residue with 50 c.c. warm water.

EXPT. 39. *Preparation of Emulsin from Sweet Almonds*

Remove the testas from a dozen sweet almonds, chop the almonds finely and cover with ether in a flask for about 10 minutes. Decant the ether and discard, and repeat twice the steeping with ether to remove most of the fat. Pound the residue in a mortar with distilled water, filter, and repeat until about 100 c.c. of emulsin extract are obtained.

Expt. 40. *Hydrolysis of Amygdalin by Emulsin*

In one test-tube place equal volumes of amygdalin and emulsin solutions, in another amygdalin solution and well-boiled emulsin solution. Insert sodium picrate papers with the corks. The former mixture will be found to liberate hydrocyanic acid, in contrast to the second, where the enzyme has been inactivated by heat.

The enzyme **prunase** is present without amygdalase in the leaves of many species of *Prunus*, and three **mandelonitriles** occur in nature as β-glucosides. The glucoside of the *dextro*-rotatory form is, as we have seen, obtainable from amygdalin; it occurs also as **prunasin** in young branches of the Bird Cherry (*Prunus Padus*) and bark of the Wild Cherry (*Prunus Cerasus*). The glucoside of the *laevo*-form is **sambunigrin,** found in the leaves of the Elder (*Sambucus nigra*), while the glucoside of the inactive (*dl*-) form is **prulaurasin** of Cherry Laurel leaves (*Prunus Laurocerasus*).

Vicianin, in the seeds of the Vetch (*Vicia angustifolia*), corresponds to amygdalin, in which one molecule of glucose is replaced by arabinose, giving the disaccharide vicianose in place of gentiobiose. The seeds also contain an enzyme **vicianase,** which hydrolyses off the disaccharide.

Phaseolunatin (linamarin) occurs in seeds of *Phaseolus lunatus*, in seedlings of Flax (*Linum usitatissimum*), and in seeds of the Rubber Tree (*Hevea braziliensis*). It is acetone-cyanhydrin-glucoside, $C_6H_{11}O_5—O—C(CH_3)_2CN$, and gives glucose, acetone, and hydrocyanic acid on hydrolysis. A specific enzyme, **linase,** accompanies it in the flax plant.

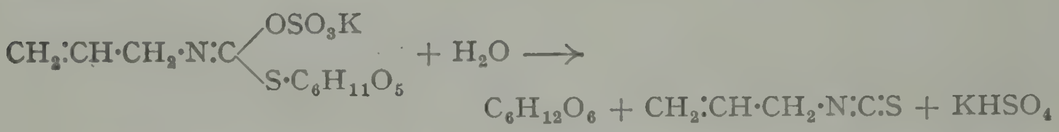
Lotusin from *Lotus arabicus* is interesting in that it gives on hydrolysis, besides gentiobiose and hydrocyanic acid, a yellow pigment lotoflavin (p. 205).

II. The Mustard Oil Glycosides

The mustard oil glycosides are *sulphur*-containing substances, found chiefly in the *Cruciferæ*. On hydrolysis they yield **mustard oils,** which are organic compounds containing the *iso*thiocyanate ($—N{=}C{=}S$) grouping, and having a characteristic odour. An enzyme **myrosin** accompanies them in the plant, and is contained in special cells. The following are the best-known members of this group:—

Glycoside	*Source*	*Hydrolysis Products*
Sinigrin	*Brassica nigra*	Glucose + allyl *iso*thiocyanate + $KHSO_4$
Gluconapin	*Brassica Napus*	Glucose + crotonyl *iso*thiocyanate
Glucocheirolin	*Cheiranthus*	Glucose + cheirolin
Glucotropæolin	*Lepidium, Tropæolum*	Glucose + benzyl *iso*thiocyanate + $KHSO_4$
—	*Nasturtium*	Glucose + phenylethyl *iso*thiocyanate
Sinalbin	*Brassica alba*	Glucose + *p*-hydroxybenzyl *iso*thiocyanate + acid sinapin sulphate

Sinigrin, $C_{10}H_{16}O_9NS_2K$, is the simplest of the mustard oil glycosides. It occurs not only in Black Mustard seed, but also in other species of *Brassica*, and in the root of the Horseradish (*Cochlearia Armoracia*). The enzyme **myrosin** also occurs in the seed, and hydrolyses the glucoside to glucose, allyl *iso*thiocyanate (or mustard oil), and potassium bisulphate, according to the equation:



$$CH_2{:}CH{\cdot}CH_2{\cdot}N{:}C\left\langle\begin{array}{l}OSO_3K\\ S{\cdot}C_6H_{11}O_5\end{array}\right. + H_2O \longrightarrow C_6H_{12}O_6 + CH_2{:}CH{\cdot}CH_2{\cdot}N{:}C{:}S + KHSO_4$$

A related compound, *viz.* **allyl sulphide,** $(CH_2{:}CH{\cdot}CH_2)_2S$, is responsible for the odour and flavour in onions and garlic.

Gluconapin from Rape seed (*Brassica Napus*) gives on hydrolysis the next higher homologue of mustard oil, *viz.* crotonyl *iso*thiocyanate, $CH_3{\cdot}CH{:}CH{\cdot}CH_2{\cdot}N{:}C{:}S$.

Glucocheirolin in Wallflower seeds is a glucoside of an aliphatic *iso*thiocyanate called **cheirolin,** which also contains a *sulphone* grouping (SO_2), and has the formula $CH_3SO_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}N{:}C{:}S$. The next higher homologue occurs as a glucoside in *Erysimum.*

Several aromatic *iso*thiocyanates are produced by the hydrolysis of glucosides.

Glucotropæolin in the garden Nasturtium (*Tropæolum majus* of the *Geraniaceæ*) and in several Cresses (*e.g. Lepidium sativum*) gives benzyl *iso*thiocyanate, $C_6H_5{\cdot}CH_2{\cdot}N{:}C{:}S$, on hydrolysis; while Water Cress (*Nasturtium officinale*) gives the next higher homologue, phenylethyl mustard oil.

Sinalbin, $C_{30}H_{42}O_{15}N_2S_2$, from White Mustard seed, is much more complex than sinigrin. On hydrolysis with myrosin it yields glucose, *p*-hydroxybenzyl *iso*thiocyanate (or sinalbin mustard oil), $C_6H_4(OH){\cdot}CH_2{\cdot}N{:}C{:}S$, and acid sinapin sulphate, $C_{16}H_{24}O_5N{\cdot}HSO_4$. This last substance on treatment with baryta gives the nitrogenous compound **choline** (p. 129) and the aromatic acid, **sinapinic acid,** $C_6H_2(OH)(OCH_3)_2{\cdot}CH{=}CH{\cdot}COOH$, chemically related to cinnamic acid (p. 181).

III. THE SAPONINS

The saponins form a group of glycosides widely distributed in plants, more especially in the roots, leaves, and seeds; they have been detected in more than 400 plants, belonging to about 70 natural orders. The most characteristic property of these glycosides is their ability to form *colloidal solutions* in water which give a soapy foam and form stable emulsions with oils, fats, and resins. The Soapworts (*Saponaria*) and the Soapnuts (*Sapindus*) owe their names to this property. Such solutions also have a marked power of occluding gases; the non-toxic saponin, sarsaparilla, is used commercially in the manufacture of carbonated beverages. Most saponins are toxic, especially to cold-blooded creatures such as fish; many of them also give characteristic additive compounds with cholesterol, which are poisonous. In addition, the *Digitalis* and *Strophanthus* glucosides form a sub-group sometimes called the **cardiac glycosides,** because of their effect on the heart. The saponins are usually light-coloured powders, a few of them being crystalline. They are isolated by extraction with water or alcohol from the plant tissue, followed by precipitation with lead acetate solution. Most saponins give a red or violet coloration with concentrated sulphuric acid.

On hydrolysis with mineral acid they yield a variety of sugars, including glucose, galactose, arabinose, and some unique pentose sugars, *e.g.* digitoxose and digitalose (p. 73), together with **sapogenins,** which are physiologically active.

The Cardiac Saponins. 'Digitalis' is the pharmacological name for the mixture of closely related glycosides isolated from the *leaves* of the Foxglove (*Digitalis*). The *seeds*, on the other hand, give 'digitalinum germanicum', a mixture of different but still closely related glycosides. The leaf glycosides increase in the youngest leaves until they form about 1 per cent. of the total dry matter, while the seed glycosides disappear on germination, the products being converted into the leaf glycosides.

The C_{41}-glycosides **digitoxin, gitoxin,** and **digoxin** are the chief products from the leaves, while **digitalin** is obtained from the seeds. Recently Stoll has shown that these glycosides are not the original substances present in the plant tissue. In *Digitalis lanata* the original glycosides, **digilanides A, B,** and **C,** are accompanied by an enzyme *digilanidase* which removes one molecule of glucose and one of acetic acid to give the C_{41}-glycosides. Extraction methods which inhibit this enzymatic hydrolysis give the digilanides themselves. The C_{41}-compounds are termed **progenins.** These in turn can be hydrolysed by dilute acid to give the rare pentose sugar, **digitoxose,** and the **C_{23}-genins** as follows:—

Natural Glycoside	$\xrightarrow[\text{enzyme}]{}$	Glucose + Acetic Acid + *Progenin*	$\xrightarrow[\text{acid}]{}$	3 Mols. Digitoxose + *Genin*
Digilanide A $C_{49}H_{76}O_{19}$		Digitoxin $C_{41}H_{64}O_{13}$		Digitoxigenin $C_{23}H_{34}O_4$
Digilanide B $C_{49}H_{76}O_{20}$		Gitoxin $C_{41}H_{64}O_{14}$		Gitoxigenin $C_{23}H_{34}O_5$
Digilanide C $C_{49}H_{76}O_{20}$		Digoxin $C_{41}H_{64}O_{14}$		Digoxigenin $C_{23}H_{34}O_5$

Similarly *Digitalis purpurea* contains **purpurea glycosides A** ($C_{47}H_{74}O_{18}$) and **B** ($C_{47}H_{74}O_{19}$) which on hydrolysis yield respectively two of the same progenins, digitoxin and gitoxin, and one molecule of glucose. The seed glycoside **digitalin** is hydrolysed to glucose, **digitalose,** and dianhydro-gitoxigenin.

The **Strophanthins** occur in members of the *Apocynaceæ.* Seeds of various species of *Strophanthus,* used in the tropics as arrow poisons, contain the glycoside **strophanthin,** which on hydrolysis with acid yields glucose, cymarose, and strophanthidin. **Cymarose** is a 3-methyl ether of digitoxose. **Cymarin** from Indian Hemp (*Apocynum cannabinum*) yields cymarose and strophanthidin. An enzyme, *strophanthobiase,* present in the seeds of *Strophanthus courmonti,* effects a partial hydrolysis of strophanthin to cymarin with elimination of the glucose only. Another glycoside of the same type is **convallatoxin** from the blossoms of the Lily of the Valley (*Convallaria majalis*). This is a rhamnoside of strophanthidin, but the rhamnose linkage is so much harder to break by acid hydrolysis than in the 2-desoxy sugars cymarose and digitoxose, that the strophanthidin is decomposed to an anhydro-derivative. This is also exemplified by the acid hydrolysis of digitalin (*vide supra*).

Scillaren from the Sea Onion or Mediterranean Squill (*Scilla maritima*) was used by ancient Egyptians and Romans. It is a mixture of at least two glycosides. Scillaren A on acid hydrolysis yields glucose, rhamnose and **scillaridin A.**

Strophanthidin, the *Digitalis* genins, and scillaridin are all **steroids** (p. 55). Digitoxigenin is the simplest compound of the group. It contains the sterol ring structure (the aliphatic side-chain on carbon atom number 17 of the sterols is replaced with a lactone five-membered ring side-chain). In scillaridin this side-chain becomes a six-membered ring. In all cases, the sugar residue is attached to the hydroxyl group of ring A, and where more than one molecule of sugar is present, these are condensed with each other to form a polysaccharide unit attached to this hydroxyl group.

The Non-Cardiac Saponins. The non-cardiac saponins fall into two groups according to the structure of their aglucones or sapogenins, *viz.* (i) the steroid sapogenins, and (ii) the triterpenoid sapogenins.

(i) The most studied of the **steroid saponins** occur along with the cardiac glycosides in *Digitalis* seeds. The following table shows their composition and acid hydrolytic products:—

Saponin	*Sapogenin*	*Sugars*
Tigonin $C_{56}H_{92}O_{27}$	Tigogenin $C_{27}H_{44}O_3$	2 mols. glucose, 2 mols. galactose, 1 mol. xylose
Gitonin $C_{50}H_{82}O_{23}$	Gitogenin $C_{27}H_{44}O_4$	3 mols. galactose, 1 mol. pentose
Digitonin $C_{56}H_{92}O_{29}$	Digitogenin $C_{27}H_{44}O_5$	4 mols. galactose, 1 mol. xylose

These 'digitalis' saponins are non-toxic except on administration intravenously. They hæmolyse red blood corpuscles in very low concentrations, and as they are more toxic to lower animal forms, they have been used extensively as fish poisons. **Sarsasaponin,** $C_{47}H_{74}O_{17}$, or 'sarsaparilla' occurs in the dried root of the Smilax (*Smilax*). It gives on hydrolysis 2 mols. glucose, rhamnose, and sarsasapogenin, $C_{27}H_{44}O_3$, which is a steroid. **Dioscin** (from *Dioscoria*) and **trillin** and **trillarin** (from *Trillium erectum*) on hydrolysis all give diosgenin; this has been converted into the hormone progesterone and also into tigogenin, showing the presence of the same steroid nucleus. Other sapogenins obtained by acid hydrolysis of naturally occurring saponins, and which have been identified, are **smilagenin,** $C_{27}H_{44}O_3$, from the saponin of Jamaica sarsaparilla root, **chlorogenin,** $C_{27}H_{44}O_4$, from *Chlorogalum,* and *lilligenin,* $C_{27}H_{44}O_4$, from *Lillium rubrum.* Related to these are several steroid alkaloids (p. 227) from the *Solanaceæ.* The glycosidic **solanines** are hydrolysed to solanidines, steroids with a tertiary amino group.

(ii) The **triterpenoid saponins** show the characteristic foaming action in water and the hæmolytic effect on red blood corpuscles. Hydrolysis removes the glycosidic sugar and leaves sapogenins, which on dehydrogenation yield a mixture of **aromatic hydrocarbons.** Chief among these are *sapotalene,* which is 1, 2, 7-trimethyl naphthalene (p. 178) and 1, 8-dimethyl picene, $C_{24}H_{18}$. This latter compound consists of five condensed benzene rings; it is, however, also related to the steroids, as picene can be obtained from cholic acid by dehydrogenation at high temperature. The chief saponins of this group include **hederin,** $C_{41}H_{64}O_{11}$, which occurs in the Ivy (*Hedera Helix*). Hydrolysis yields rhamnose, arabinose, and hederagenin, $C_{30}H_{48}O_4$, which is also obtained from the saponin of Soapnuts (*Sapindus* sp.). **Saporubin,** from the root of the Soapwort (*Saponaria officinalis*), and **Levant sapotoxin,** from roots of *Gypsophila* species (both *Caryophyllaceæ*) yield the sapogenin gypsogenin, $C_{30}H_{46}O_4$.

IV. THE PHENOLIC, COUMARIN, AND HYDROXYANTHRAQUINONE GLYCOSIDES

The aglucones of these glycosides are all aromatic compounds containing a phenolic grouping (—OH) to which the sugar residue is attached. The aglucones may be simply phenols, or may in addition be alcohols, aldehydes, ketones, esters, etc. The structure of many of these is discussed with the aromatic compounds in Chapters XVII and XVIII. An account of the chief glycosides of this group and of their occurrence follows here.

Arbutin, $C_{12}H_{16}O_7$, gives on hydrolysis glucose and the phenol called hydroquinone (p. 176). It occurs in the *Ericaceæ*, especially in leaves of the Bearberry (*Arctostaphylos Uva-ursi*), and is also present in the leaves, bark, and roots of many varieties of Pear (*Pyrus communis*). Associated with it in both the Bearberry and some Pears is **methylarbutin,** which gives glucose and methylhydroquinone on hydrolysis. Leaves of some varieties of Pear turn black when they fall; this is due to the hydrolysis of the arbutin and the subsequent oxidation of hydroquinone, whereas leaves containing methyl-arbutin as well give first a yellow oxidation product. Arbutin can be hydrolysed by emulsin.

Gein, which occurs in the Common Avens (*Geum urbanum*), gives on hydrolysis with mineral acid, or with the enzyme **gease** associated with it in the plant, the disaccharide, vicianose and the methoxyphenol, eugenol (p. 177).

Phloridzin on hydrolysis yields glucose and the complex phenolic substance, phloretin, $C_6H_2(OH)_3 \cdot COCH_2CH_2 \cdot C_6H_4OH$, related to phloroglucinol (p. 177). It occurs in the bark, especially in the root-bark, of species of *Pyrus*, *viz.* Apple and Pear, and *Prunus*, *viz.* Cherry and Plum, and also in leaves of the Apple. It is used in experimental animal physiology as it produces severe glycosuria (the inability to utilise the blood sugar) when injected into animals. **Glycophyllin,** which occurs in *Smilax Glycophylla*, gives phloretin and rhamnose on hydrolysis.

Salicin, $C_{13}H_{18}O_7$, on hydrolysis gives glucose and the phenolic alcohol saligenin, or salicylic alcohol (p. 181). It occurs in the bark, leaves, and female flowers of many species of *Salix*, in the bark of the Poplar (*Populus*), and in the flower-buds of the Meadowsweet (*Spiræa Ulmaria*). The corresponding specific enzyme **salicase** occurs in the leaves and twigs of the Willow, but emulsin can also effect this hydrolysis.

EXPT. 41. *Hydrolysis of Salicin by Emulsin*

In two test-tubes place (*a*) equal volumes of an aqueous solution of salicin and of the emulsin solution from the experiment on p. 104, (*b*) salicin solution and emulsin solution which has been previously boiled. Allow the tubes to stand for a few hours, then add a few drops of ferric chloride solution to each; a purple colour appears in (*a*) owing to the formation of salicylic alcohol.

Populin, $C_{20}H_{22}O_8$, or monobenzoyl-salicin, on hydrolysis yields benzoyl-glucose and salicylic alcohol, showing that the benzoyl group is present in the sugar nucleus. Populin occurs with salicin in the bark of various species of Poplar.

Coniferin, $C_{16}H_{22}O_8$, is hydrolysed by emulsin or acids to glucose and the phenolic alcohol, coniferyl alcohol (p. 183). Coniferin is widely distributed in the bark of *Coniferæ*, and also in Beet, *Scorzonera*, and Asparagus. **Syringin,** $C_{17}H_{24}O_9$, or methoxy-coniferin, is hydrolysed by emulsin to glucose and syringenin or 5-methoxy-coniferyl alcohol, which is the alcohol corresponding to sinapinic acid in the glucoside sinalbin (p. 106). It occurs in the *Oleaceæ*, *e.g.* in species of Lilac (*Syringa*), Jasmine (*Jasminum*), and Privet (*Ligustrum*).

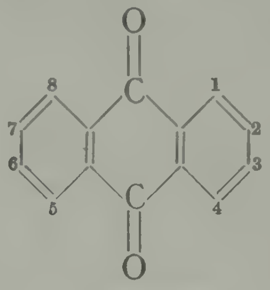
Spiræin, $C_{13}H_{16}O_7$, is the glucoside of a phenolic aldehyde, salicylaldehyde (p. 181), and occurs in the roots of *Spiræa Ulmaria* and *S. kamschatica*.

Picein, $C_{14}H_{18}O_7$, salinigrin, or salicinerin, occurs in several species of *Salix* and *Populus*, and on hydrolysis yields glucose and a hydroxy-ketone, *p*-hydroxyacetophenone, $HO{\cdot}C_6H_4{\cdot}CO{\cdot}CH_3$.

Gaultherin, $C_{19}H_{26}O_{12}$, on hydrolysis yields the ester, methyl salicylate (p. 181) and the disaccharide, primeverose (p. 75). It occurs in roots of species of *Gaultheria* and *Spiræa*, and a specific enzyme **gaultherase** is associated with it in the former plants. It appears to be identical with the glycoside called **monotropin** in *Monotropa Hypopithys* and *Betula lenta*. The glycoside of methyl salicylate with the disaccharide vicianose is **violutin,** $C_{19}H_{26}O_{12}$, in *Viola cornuta*.

The **coumarin glycosides** are discussed with coumarin (p. 183); the most common are **æsculin** in Horse Chestnut bark (*Æsculus Hippocastanum*) and **daphnin** in various species of *Daphne*.

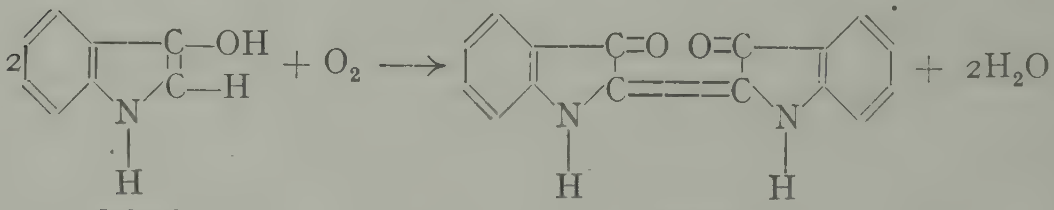
The **hydroxyanthraquinone glycosides** are derived from the parent compound anthraquinone, $C_{14}H_8O_2$, containing two benzene rings linked through a diketo-ring, as follows:—



Anthraquinone

The numbers refer to the possible position of hydroxyl groups, to one of which the sugar molecule is attached. Such substances are widely distributed in plants, both free and as glycosides, and some of the plant extracts which were important in the dyeing industry before the preparation of synthetic dyes belong to this group. **Madder,** which consists of the ground root of *Rubia tinctorum*, contains a mixture of glycosides, the principal one being **ruberythric acid,** $C_{25}H_{28}O_{14}$, which on hydrolysis yields primeverose (p. 75) and 1, 2-dihydroxyanthraquinone or **alizarin.** A widely distributed aglucone is **emodin** or 4, 5, 7-trihydroxy-2-methyl-anthraquinone, which occurs in Rhubarb (*Rheum*); it also occurs as the rhamnoside **frangulin** in the bark of *Rhamnus Frangula*, and as the glucoside **polygonin** in *Polygonum cuspidatum*. The latter also contains a glucoside of emodin-dimethylether.

Another important natural dyestuff, **indigo,** occurs as a colourless glucoside **indican** in the Indigo plants (*Indigofera*), in the Woad plant (*Isatis tinctoria*), in *Polygonum tinctorium*, and in several of the *Orchidaceæ, e.g. Calanthe* and *Phajus*. Indican is hydrolysed by an enzyme **indemulsin** occurring in the plant, or by dilute acid, to give glucose and indoxyl, a heterocyclic compound containing nitrogen. Indoxyl is colourless, but is easily oxidised by atmospheric oxygen (a process catalysed in the plant by an oxidising enzyme or *oxidase*) to **indigotin,** which is the deep blue dye known as indigo.



Indoxyl

Indigotin

Physiological Function of the Glycosides

With such a variety of compounds, whose only similarity is that they contain a sugar residue in the molecule, it is not to be expected that they will all have the same function in the plant. Some of the possible functions are as follows:—

(1) *Reserve Food Material.* Some glycosides have a definite seasonal variation, often accumulating in autumn and winter, and disappearing in spring when more active metabolism sets in. Salicin and arbutin are both of this type. An examination of the content of salicin in many species of Willow and Poplar has shown that it not only varies from one species to another, but between male and female plants of the same species. In one instance, the bark of the female plant contained three times as much salicin in April as the male, whereas in July the conditions were reversed (Jowett and Potter). It would appear that at least the glucose, and probably also the salicylic alcohol, since it does not accumulate, are both used as reserve material. The seasonal difference in glycoside content is important in the case of cyanophoric glycosides in plants of pastures and in weeds. Several plants are toxic to animals when young, owing to a high glycosidal content, *e.g.* species of *Lotus* containing lotusin, and the Millet containing dhurrin, both of which are absent in the mature plant.

(2) *Removal of Injurious Substances.* This is probably the most general function of the glycosides, namely, the conversion of aglucones which are insoluble, or chemically active in the free state so that they are susceptible to oxidation and polymerisation reactions, into soluble, chemically stable and inert substances. A similar function is played in the animal system by the uronic acids, glucuronic acid derivatives of such substances as camphor being excreted in the urine. Plants are also able to form glycosides from substances which are not naturally present in their cell-sap. Exposure of Potatoes to ethylene chlorohydrin led to the synthesis of a glucoside, whereas exposure of Gladiolus corms and Tomato plants of chloral hydrate and *o*-chlorophenol resulted in the formation of the corresponding gentiobiosides (Miller).

(3) *Antiseptics and Hormones.* As the corresponding enzyme is so often adjacent to the glycoside in the plant, and as many of the aglucones are antiseptic and bactericidal, their presence in seeds of plants and bark of trees may effect some control of invasion by disease on injury, and may even be one of the deciding factors in immune varieties. For instance, both benzaldehyde and hydroquinone inhibit the growth of fungi, while the presence of the

glucoside solanin, resembling the saponins, has been correlated with the immunity of potatoes to dry rot. Again, some of the aglucones, such as hydroquinone, are easily oxidised substances, and therefore may act as oxygen carriers, and thereby affect the rate of respiration. Other aglucones may activate enzymes. Hence the liberation of these aglucones in the living plant may induce more active metabolism in the tissue concerned. This would parallel the action of hormones in animals, and it has recently been shown that regulatory substances with similar functions do exist in plants (p. 305).

(4) *Biological Function.* It has been indicated that a relatively large number of the glycosides are not only toxic to animals, but also readily liberate hydrocyanic acid or mustard oils. It is therefore obvious that those glycosides which occur in immature fruits and seeds, and disappear or diminish when ripening is complete, perform a biological function in protecting the plant from raids by animals until the appropriate time arrives for the distribution of the seed.

PART IV. PLANT ACIDS

CHAPTER XII

POLYBASIC AND HYDROXY-ACIDS

THE reaction of the cell-sap of most plants is on the acid side of neutrality; some plants have extremely acid cell-saps, while some tissues such as unripe fruits and leaves of Sorrel are acidic even to the taste. This acidity is due to the presence of organic acids, which must be distinguished from the fatty acids already described, as the latter, with the exception of one or two of the simpler members such as *iso*valeric acid, occur in the plant mainly in the *combined form* as fats and oils or simpler esters. The plant acids—that is, acids existing in the plant in the free state—are all *acids of multiple function*; they contain more than one reactive group, which may be the same, *viz.* two carboxyl groups, or different, *viz.* a carboxyl and a hydroxyl group. A few members of this last type, the **hydroxy-acids,** also occur in the fats and oils (p. 32). The plant acids are relatively simple in structure; they include **dibasic acids** and **hydroxy-acids,** and most of them give sparingly soluble calcium salts, a property which is used in the standard method of isolating the acids from plant tissues.

DIBASIC ACIDS

There is a homologous series of dibasic acids. The members are solids, with high melting-points; they are readily soluble in water, and possess greater acidity than the corresponding fatty acids because of the two carboxyl groups. The dibasic acids, like inorganic dibasic acids, can form either acid or normal salts. The following acids of the dibasic series have been found in plants:—

Oxalic acid	$HOOC{\cdot}COOH$
Malonic acid	$HOOC{\cdot}CH_2{\cdot}COOH$
Succinic acid	$HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$
Glutaric acid	$HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$
Adipic acid	$HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$

Oxalic Acid, $(COOH)_2$

Occurrence. Oxalic acid is very widely distributed in plants, occurring mainly as the insoluble **calcium salt** in the form of microscopic crystals (rhapides) in plant cells and in cell-walls, and to a less extent as the more soluble **sodium** and **potassium salts** in the cell-sap. It is especially abundant in the leaves of species of *Oxalis* (of the *Geraniaceæ*), *e.g.* Wood-sorrel (*Oxalis Acetosella*); in the leaves of *Begonia*; in members of the *Polygonaceæ*, such as leaves and stems of Rhubarb (*Rheum Rhaponticum*), leaves of the Sorrel (*Rumex Acetosa*), of Sheep's Sorrel (*Rumex Acetosella*), and of the Mountain Sorrel (*Oxyria digyna*); and in species of *Mesembryanthemum.* Calcium oxalate is deposited to the extent of 20 per cent. in the barks of certain species of *Eucalyptus*, and large quantities occur in the bark of a Himalayan tree, *Shorea robusta.* Potassium hydrogen oxalate occurs in the Wood-sorrel, from which it was first isolated. Oxalic acid appears to be the end-product of acid formation in the plant (p. 123); in some cases it is elaborated when an excess of bases, especially calcium, must be removed from solution in the cell-sap.

Properties. Oxalic acid is a colourless, crystalline solid, which dissolves in water to give a solution acid to litmus; this solution liberates carbon dioxide from sodium carbonate. Calcium oxalate, $(COO)_2Ca$, is insoluble in dilute acetic acid, even on warming, but is soluble in dilute hydrochloric acid. This behaviour can be used microchemically to identify calcium oxalate crystals in plant tissues. Oxalic acid and soluble oxalates are poisonous, and milk of lime is used as an antidote. Potassium hydrogen oxalate, $HOOC{\cdot}COOK$, and the double salt, potassium quadroxalate, $HOOC{\cdot}COOK, (COOH)_2, 2H_2O$, are both known as 'salts of sorrel' and 'salts of lemon'; the former is used as a mordant in dyeing, and the latter in bleaching. Some of the principal reactions of oxalic acid are demonstrated by the following experiments:—

Expt. 42. *Reactions of Oxalic Acid*

1. Heat oxalic acid in a dry test-tube. Carbon monoxide is evolved, and will burn at the mouth of the test-tube.

2. Heat oxalic acid with concentrated sulphuric acid. CO and CO_2 are evolved, but no charring occurs.

3. Show that oxalic acid gives effervescence of CO_2 with sodium carbonate solution.

4. Show that oxalic acid is oxidised by potassium permanganate solution in the presence of dilute sulphuric acid.

5. Add calcium chloride solution to a cold *neutral* solution (see p. 28) of oxalic acid, and note that a white precipitate forms. Show that it is insoluble in acetic acid, but soluble in hydrochloric acid.

Expt. 43. *Preparation of Calcium Oxalate and of Oxalic Acid from Leaves of Sorrel or Rhubarb*

Take 200 grm. of fresh leaves and boil them with sufficient water to cover them. Squeeze through muslin, boil the extract again, then filter through filter-paper. Acidify the filtrate with acetic acid, and add a concentrated solution of calcium acetate until no more precipitate is formed. Allow the precipitate to settle, then decant off the supernatant liquid. Dissolve the precipitate in the minimum amount of boiling dilute hydrochloric acid, and crystals of calcium oxalate will separate on cooling.

Dry and weigh some of the crystals and add the calculated amount of sulphuric acid (1 grm. of calcium oxalate requires 15·6 c.c. *N*-acid); stir well, allow to stand for 10 minutes, then filter off the calcium sulphate. Evaporate the filtrate to small bulk on a water-bath, when crystals of hydrated oxalic acid will separate on cooling $(COOH)_2, 2H_2O$.

Malonic acid, $CH_2(COOH)_2$, occurs in the Sugar Beet (*Beta vulgaris*) as the **calcium salt,** and is also present in Wheat, Barley, and Oats. In the form of its ethyl ester, malonic acid is important in synthetic organic chemistry.

Succinic acid, $(CH_2{\cdot}COOH)_2$, is widely distributed in plants, especially in *unripe fruits*, and has been isolated from Grapes (*Vitis vinifera*), Gooseberries and Currants (*Ribes*), Apples (*Pyrus Malus*), and Bananas (*Musa*). It has also been obtained from Rhubarb leaves and stems, and from Lettuce (*Lactuca*). A **basic aluminium succinate** occurs as a deposit in the timber of the Australian tree, *Orites excelsa*. Succinic acid was first prepared by the dry distillation of the fossil 'amber' (Lat. *succinum*), and it also occurs in fossilised wood. It is a colourless, crystalline solid, and is best isolated as the insoluble **barium** salt; the calcium salt is only precipitated from concentrated solutions or on addition of alcohol.

Expt. 44. *Reactions of Succinic Acid*

1. Heat succinic acid in a dry test-tube; suffocating fumes of the anhydride are evolved.

2. To a neutral solution of succinic acid add ferric chloride solution. A dense reddish-brown precipitate of ferric succinate is formed, discharged by hydrochloric acid to give a clear solution.

3. Silver nitrate solution gives with succinic acid solution an insoluble precipitate of silver succinate, unaltered by heating.

4. Add barium chloride solution to a solution of succinic acid; the barium salt is precipitated.

Glutaric acid, $CH_2{\cdot}(CH_2{\cdot}COOH)_2$, and **adipic acid,** $(CH_2{\cdot}CH_2{\cdot}COOH)_2$ have been isolated together with malonic acid from the Sugar Beet. It will be observed that a wide variety of compounds have been detected in the Beet, owing to the examination of the residues from beet sugar manufacture. Many other plant tissues would in all probability show a similar complexity of contents if they were investigated as completely.

Hydroxy-acids

With the exception of the first substance discussed, glycollic acid, the plant hydroxy-acids are also dibasic and even tribasic, whereas the hydroxy-acids in the fats and oils (p. 32) are long-chain monobasic acids. The hydroxy-acids discussed below are distinguished as a group by giving a yellow coloration with ferric chloride in neutral solution.

Glycollic acid, $CH_2(OH)\cdot COOH$, or *hydroxy-acetic acid*, is the simplest monobasic hydroxy-acid and occurs in unripe Grapes (*Vitis vinifera*). It also occurs in the Tomato (*Solanum Lycopersicum*), in the Sugar-cane (*Saccharum*), in leaves of the Virginian Creeper (*Ampelopsis*), and in Alfalfa (*Medicago*). Glycollic acid is a colourless, crystalline solid. As in the alcohols, the primary alcoholic group can be oxidised first to an aldehydic and then to an acidic group. Glycollic acid therefore may be oxidised first to glyoxalic acid, $CHO\cdot COOH$, and then to oxalic acid, $COOH\cdot COOH$. **Glyoxalic acid** is also common in unripe fruits.

Lactic acid, $CH_3\cdot\mathbf{C}H(OH)\cdot COOH$, or *α-hydroxy-propionic acid*, is a low-melting crystalline solid (m.p. 18° C.), usually obtained as a viscid liquid. The molecule contains one asymmetric carbon atom (in heavy type), and therefore can be obtained in three forms, two optically active and one inactive. *dl*-Lactic acid, the last-mentioned form, is produced by the action of bacterial ferments on lactose and other sugars, and occurs in sour milk and in cheese. *d*-Lactic acid occurs in muscle, and is of importance in animal respiration.

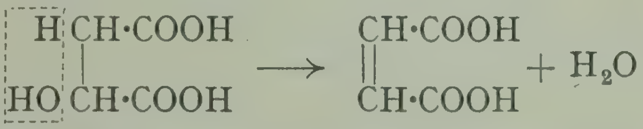
Pyruvic acid, $CH_3\cdot CO\cdot COOH$, is the ketonic acid corresponding to lactic acid. It is one of the key three-carbon metabolites in both plant and animal mechanisms for the breakdown of carbohydrate in respiratory processes (p. 280).

Keto-Enol Tautomerism. A characteristic property of compounds containing the ketonic arrangement, $>HC{-}C{=}O$, is that many of the reactions can only be explained by assuming the transfer of the hydrogen atom to give a hydroxyl group, $>C{=}C{-}OH$. This is called the **enol** form, and will be encountered in discussions on the sugars (p. 68) and on the mechanism of fermentation and respiration. Pyruvic acid furnishes an important instance:—

$$CH_3\cdot CO\cdot COOH \rightleftharpoons CH_2{=}C(OH)\cdot COOH$$

Malic Acid, $HOOC\cdot\mathbf{C}H(OH)\cdot CH_2\cdot COOH$

Malic acid, or *hydroxy-succinic acid*, contains one asymmetric carbon atom in the molecule; only the *laevo*-isomer occurs in nature. It is widely distributed in plants, especially in unripe fruits such as those of Apples, Pears, Gooseberries, and Mountain Ash berries, also in the leaves and vegetative parts of several of the *Crassulaceæ*, of several *Cactaceæ*, of *Begonia*, and of *Mesembryanthemum*. In these plants it occurs as the free acid. Potassium hydrogen malate has been isolated from stalks of Rhubarb and the fruit of Currants (*Ribes*), while the calcium salt is present in the sap of various species of Maple (*Acer*). *l*-Malic acid forms colourless crystals which deliquesce readily; it can be isolated as the calcium salt, which is precipitated almost quantitatively from aqueous alcohol. When heated, malic acid undergoes dehydration, and a sublimate is obtained consisting of a mixture of two unsaturated acids, *maleic* and *fumaric* acids:—



$$\begin{array}{l}\text{H}\ \text{CH·COOH}\\ \qquad |\\ \text{HO}\ \text{CH·COOH}\end{array} \longrightarrow \begin{array}{l}\text{CH·COOH}\\ \|\\ \text{CH·COOH}\end{array} + H_2O$$

These two acids differ in the orientation of the hydrogen and carboxyl groups round the rigid double bond; maleic acid has the two carboxyl groups on the same side of the bond (*cis*-position) and can form an anhydride, while fumaric acid has the acid groups in the *trans*-position. **Fumaric acid** occurs in plants, being present in relatively high proportions in the *Fumariaceæ*, in *Glaucium*, and in many fungi. Fumaric acid and similar unsaturated dibasic acids are possible intermediate compounds in respiration (p. 283), and in the formation of the plant amino-acids.

EXPT. 45. *Reactions of Malic Acid*

1. Heat malic acid in a dry test-tube; it melts, and fumes of maleic acid condense in white crystals on the cooler parts of the tube.

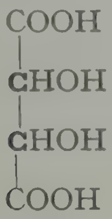
2. Sodium carbonate solution gives effervescence of carbon dioxide with malic acid.

3. Show that a neutral solution of malic acid gives only a yellow coloration with ferric chloride solution.

4. To a dilute neutral solution of malic acid add calcium chloride solution. Boil, then cool, and add an equal volume of alcohol. Calcium malate is precipitated. Show that it is soluble in acetic acid.

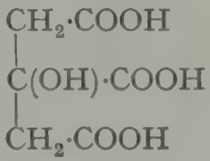
EXPT. 46. *Preparation of Malic Acid from Apples*

Put about 500 grm. of unripe apples rapidly through the mincer, mash with a little water in a mortar, then squeeze through muslin. Boil the juice, filter, and then to the boiling filtrate add calcium carbonate till no further effervescence takes place. Cool the solution, and add twice its volume of alcohol to ensure complete precipitation of the calcium malate. The latter is filtered off, washed with alcohol, dried, and weighed. Add the calculated amount of dilute sulphuric acid (1 grm. calcium malate requires 6 c.c. 2N-acid), allow the calcium sulphate to settle, then remove it by filtration. Evaporate the filtrate to small bulk, place in an evacuated desiccator; crystals of malic acid will separate.



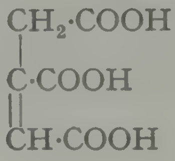
$$\begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ COOH \end{array}$$

(I)
Tartaric acid



$$\begin{array}{l} CH_2{\cdot}COOH \\ | \\ C(OH){\cdot}COOH \\ | \\ CH_2{\cdot}COOH \end{array}$$

(II)
Citric acid



$$\begin{array}{l} CH_2{\cdot}COOH \\ | \\ C{\cdot}COOH \\ \| \\ CH{\cdot}COOH \end{array}$$

(III)
Aconitic acid

Tartaric Acid, HOOC·CHOH·CHOH·COOH

As will be seen from formula (I) above, the molecule of tartaric acid, or *dihydroxy-succinic acid*, contains two similar asymmetric carbon atoms. This gives four possible forms, a *dextro-*, a *laevo-*, an equimolecular mixture of these, *viz.* the *dl*-form known as *racemic acid*; and a fourth 'internally compensated' or *meso-* form, which does not occur in nature.

d-Tartaric acid, or ordinary tartaric acid, occurs in many plant juices, especially of fruits such as the Grape, Mountain Ash, Tomato, and Pineapple (*Ananas sativus*). Its sole commercial source is 'argol,' which separates as a crystalline deposit in wine-vats during the fermentation process. On recrystallisation, argol yields 'cream of tartar,' or potassium hydrogen *d*-tartrate, while the mother-liquors from the crystallisation contain racemic acid, or *dl*-tartaric acid. These acids differ in the physical characteristics of solubility, water of crystallisation, and optical rotatory power, and in the solubilities of their salts. They can both be isolated by means of their calcium salts. Potassium sodium tartrate, or Rochelle salt, is used in the preparation of Fehling's solution. Tartaric acid resembles the sugars, which are also polyhydroxy-compounds, in giving an odour of burnt sugar when heated, and in showing reducing properties, *e.g.* with ammoniacal silver nitrate.

EXPT. 47. *Reactions of Tartaric Acid*

1. Heat tartaric acid in a dry test-tube. It chars readily and gives an odour of burnt sugar.

2. Heat a little tartaric acid with concentrated sulphuric acid; both charring and effervescence occur.

3. Show that tartaric acid liberates carbon dioxide from sodium carbonate solution with effervescence.

4. To a neutral solution of tartaric acid, add calcium chloride solution. A precipitate of calcium tartrate is formed, which is soluble in hydrochloric acid.

5. Show that ammoniacal silver nitrate solution gives a silver mirror on warming gently with tartaric acid solution.

EXPT. 48. *Tartrates*

Repeat tests 1 2, and 4 with Rochelle salt.

Show that Rochelle salt prevents the precipitation of cupric hydroxide on the addition of caustic soda to copper sulphate solution.

Citric Acid

Citric acid, formula (II), is a monohydroxy-tribasic acid occurring in the free state in plants, especially in fruits of the genus *Citrus*, *e.g.* Oranges, Lemons, Bergamots, also in Currants and unripe Gooseberries (*Ribes*), and in other acid fruits. The calcium salt also occurs in some roots, *e.g.* Beet and Potato. The commercial source of citric acid is unripe lemons, in the juice of which it occurs to the extent of about 6 per cent., and from which it was first isolated by Scheele (1784) through the sparingly soluble calcium salt. This salt differs from the calcium salts of the other plant acids by being less soluble in boiling water than in cold. Citric acid is also formed by the fermentative action of certain moulds on sugar. Citric acid forms large colourless prisms, and resembles tartaric acid in possessing reducing properties. When heated, it loses a molecule of water and yields the unsaturated tribasic acid, aconitic acid, formula (III), p. 120. **Aconitic acid** has been detected in the *Ranunculaceæ*, especially in the Monkshood (*Aconitum*).

EXPT. 49. *Reactions of Citric Acid*

1. Heat citric acid in a dry test-tube. It melts and slowly darkens, and fumes of aconitic acid are evolved.
2. Heat citric acid with concentrated sulphuric acid. Little charring takes place, but CO and CO_2, and on stronger heating, SO_2, are evolved.
3. Show that citric acid gives effervescence with sodium carbonate solution, owing to the liberation of CO_2.
4. To a solution of citric acid made neutral or slightly alkaline with ammonium hydroxide, add calcium chloride solution and heat to boiling. A white precipitate is obtained, soluble in acetic acid.
5. Show that citric acid reduces ammoniacal silver nitrate solution.

EXPT. 50. *Preparation of Citric Acid from Lemons*

The juice from three lemons is diluted with a little water, and filtered through muslin. The liquid is then boiled and neutralised by adding calcium carbonate in small portions. The precipitate of calcium citrate is filtered off by suction from the hot liquid, washed with a little boiling water, and dried. The calculated amount of dilute sulphuric acid is then added (1 grm. calcium citrate requires 8 c.c. 2N-acid). The calcium sulphate is allowed to settle, then is filtered off, and the filtrate evaporated to small bulk on the water-bath, when crystals of citric acid separate.

Physiological Functions of the Plant Acids

Acids which accumulate in plants are derived from at least two sources, namely, carbohydrates and amino-acids. In many *succulents* in desert climates, which show wide variations between day and night temperatures, there is an accumulation of acids during the night and a decrease in this acid content during the day. Many examples are to be found in the *Crassulaceæ*, and the *Cactaceæ*, *e.g.* the accumulation of malic acid in the Prickly Pear (*Opuntia*). This periodicity in acid formation is paralleled by a change in the respiratory quotient (p. 278). It is known that these acids are formed as intermediate oxidation products of carbohydrates in the respiratory process, so that at the low night temperatures partial oxidation of carbohydrate to acid may alone be possible, whereas at the higher day temperatures acid can also be oxidised. A restricted oxygen supply due to the peculiarities in the structure of succulents is also cited as a contributory cause. It would appear, however, that the occurrence of acids in such plants cannot be wholly explained on these grounds, as the acids have been shown to take part in synthetic processes with the formation of sugars, and are not simply all oxidised to carbon dioxide and water during the day.

The acids differ among themselves as to the minimum temperature at which they can be oxidised in the plant, malic acid being oxidised at lower temperatures than tartaric acid, while citric acid requires the highest temperature of the three. Now the disappearance of the acidity is one of the concomitants of fruit ripening (p. 311); this is due at least in part to utilisation of the acid in respiration, although some of it may be concerned in the synthesis of other compounds. Hence the particular acid most prevalent in the fruit will determine the minimum temperature and therefore the latitudes at which different fruits will ripen. Species of *Pyrus* such as Apples and Pears, which contain malic acid, can ripen at higher latitudes than Grapes (*Vitis*), in which tartaric acid predominates, and still higher temperatures are required for *Citrus* fruit, in which the principal acid is citric acid.

In several of the very acid plants, such as Rhubarb and Begonia, it has, however, been shown that the acids are derived by the elimination of ammonia from, or 'deamination' of, **amino-acids,** which are in turn formed by hydrolysis of the protein in the plant

(p. 294). A mixture of acids is obtained, malic acid being the first product in Rhubarb, but the greater part changes to oxalic acid, which appears to be the end-product in this reaction. When oxalic acid is deposited as calcium oxalate, especially in old leaves and wood, it is presumably prevented from entering farther into the active metabolism of the plant; but there is evidence that calcium oxalate is merely a temporary storage substance in unripe fruits and seeds, and, on ripening, the crystals disappear with the formation of acetaldehyde. Not only, however, is calcium absorbed to remove excess oxalic acid from solution in plant tissues, but also in some plants oxalic acid is developed to neutralise an excess of basic ions. This has been shown in the case of Maize (*Zea Mays*), where oxalic acid is produced when potassium nitrate is used as a source of nitrogen in cultures, while no oxalic acid appears if ammonium salts are used. This development of acid to neutralise metallic ions is not an exclusive function of oxalic acid, for all the acids play a part in regulating the acidity of the cell-sap and consequently in controlling enzyme activity in the protoplasm.

PART V

PROTEINS AND RELATED COMPOUNDS

CHAPTER XIII

AMINES. BETAÏNES

Nitrogen Compounds in the Plant

PLANT protoplasm consists of **proteins.** These are complex compounds containing nitrogen, sulphur, and sometimes phosphorus, in addition to carbon, hydrogen, and oxygen; and they comprise the largest and most important group of the plant nitrogenous compounds. There are in addition other nitrogenous substances found to a varying extent in plants, which are classed chemically as amines, amides, amino-acids, purines, and alkaloids.

The **amines** and **alkaloids** are both basic; but as the alkaloids form a special group, both in their restricted distribution in plants, and because they contain cyclic structures, they are considered with the other cyclic compounds (p. 218). The **betaïnes,** which are here discussed with the amines, form a connecting link between the two groups.

Amides are derivatives of acids in which the hydroxyl radical of the carboxyl group has been replaced by the *amino* group (NH_2); they are represented by the general formula, $R{\cdot}CO{\cdot}NH_2$. **Amino-acids** are carboxylic acids in which a hydrogen atom is replaced by the amino-group; for example, the simplest amino-acid is glycine, $CH_2(NH_2){\cdot}COOH$, derived from acetic acid. They are the units of which the protein molecule is composed, and can be obtained from proteins by hydrolysis. Amino-acids can also form amides, and the most important plant amides, asparagine and glutamine, are derived from amino-acids. *Purines* have a complex heterocyclic structure; they are often called bases, but in some reactions they actually ionise as acids. They occur in the free state in plant tissues, and are also important as components of nucleic acids, the universally distributed constituents of cell nuclei.

AMINES

Amines have a wide distribution in plants compared with the alkaloids, which are found only in one or a few closely related species, but they only occur in very small amounts. They are common in the animal kingdom as decomposition products of proteins, especially on putrefaction, and in plants they are also derived from proteins or the derived amino-acids.

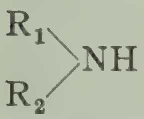
Chemically, amines are the simplest organic compounds of nitrogen. They may be regarded as derivatives of ammonia, in which one or more hydrogen atoms are replaced by organic radicals, which may be either aliphatic or aromatic. Three types of amines are therefore possible, having the following general formulæ:—

NH_3

Ammonia

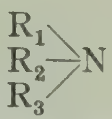
$R—NH_2$

Primary amine



$(R_1)(R_2)NH$

Secondary amine



$(R_1)(R_2)(R_3)N$

Tertiary amine

The radicals R_1, R_2, and R_3 may be the same or different. In the aliphatic series, the simplest amines are respectively (mono)-methylamine, CH_3NH_2, dimethylamine, $(CH_3)_2NH$, and trimethylamine, $(CH_3)_3N$.

Amines retain the basic character of ammonia, giving aqueous solutions alkaline to litmus, and forming salts with acids, both organic and inorganic, by a change in the valency of the nitrogen atom from three to five. For example, with hydrochloric acid, ammonia forms ammonium chloride, NH_4Cl, and methylamine forms methylamine hydrochloride, CH_3NH_2, HCl. The basicity of aliphatic amines decreases from primary to tertiary types. Amines can also act as bases in precipitating ferric hydroxide from ferric chloride solution.

The simple aliphatic amines are colourless gases or volatile liquids with an ammoniacal smell. They are soluble in water, and are therefore difficult to isolate from plants. The most widely used method is precipitation from solution with phosphotungstic acid. The action of nitrous acid on amines serves as a distinguishing test between the three types: *primary* amines evolve nitrogen, *secondary* amines give a yellow oil, a *nitrosamine*, and *tertiary* amines give no reaction.

$$RNH_2 + HNO_2 \longrightarrow ROH + N_2 + H_2O$$

$$R_2NH + HNO_2 \longrightarrow R_2N{\cdot}NO + H_2O$$

The following simple amines occur in plants:—

Monomethylamine, CH_3NH_2, a gas, is found in species of *Mercurialis*, and in the root of the Sweet-Flag (*Acorus Calamus*).

EXPT. 51. *Properties of Methylamine*

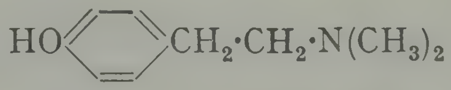
Use an aqueous solution of the gas. Test the solution with litmus, and show (*a*) that it precipitates ferric hydroxide from ferric chloride solution; (*b*) that a few drops added to copper sulphate solution give a pale blue precipitate of cupric hydroxide, while with excess a deep blue solution is produced; (*c*) that, on addition of excess dilute hydrochloric acid to the solution, followed by a concentrated solution of sodium nitrite there is evolution of nitrogen.

Trimethylamine, $(CH_3)_3N$, also a gas, occurs in the leaves of the Stinking Goosefoot (*Chenopodium vulvaria*), where it is a decomposition product of betaïne (p. 127), and in the Australian saltbush, *Rhagodia hastata.* Many flowers, *e.g.* of Hawthorn (*Cratægus Oxyacantha*) and of Mountain Ash (*Pyrus Aucuparia*), owe their peculiar odour to trimethylamine. It also occurs with monomethylamine in seeds of *Mercurialis.* Trimethylamine may occur not only as a decomposition product of betaïne, and therefore of protein, but also in the decomposition of choline (p. 129), which is a constituent of the phospholipins.

Dimethyl-, *iso*amyl-, and *iso*butyl-amines have also been identified in plants.

Putrescine, a liquid, is tetramethylene diamine, $NH_2{\cdot}(CH_2)_4{\cdot}NH_2$, and is a characteristic product of putrefying animal protein, being derived from the amino-acid, *ornithine.* Putrescine also occurs in the Thorn Apple (*Datura*); its tetramethyl derivative, $(CH_3)_2N{\cdot}(CH_2)_4{\cdot}N(CH_3)_2$, is found in one of the Henbanes (*Hyoscyamus muticus*).

Hordenine, which is *p*-hydroxyphenylethyldimethylamine, has the following structure:—



It is related to the amino-acid *tyrosine,* and has a transitory existence during the germination of Barley. It is not present in the ungerminated seed; its maximum concentration is reached four days after germination has begun, and then it diminishes and disappears by the twenty-fifth day.

Guanidine, $NH{=}C(NH_2)_2$, is also a decomposition product of proteins, and occurs in *Vicia* seedlings, and in the Sugar Beet.

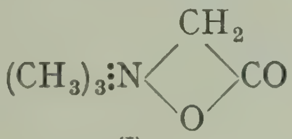
More than one hundred species of plants have been investigated by Klein for volatile amines, and it was found that the amines might be used as a chemical method of distinguishing species. Klein considered that the function of these volatile amines was to attract insects.

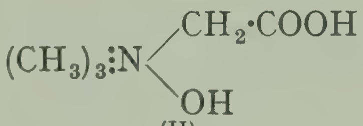
Betaïnes

The betaïnes form a natural group of simple nitrogenous compounds with feebly basic properties. They are derived from proteins, and are amino-acids in which the nitrogen atom is fully methylated. They have been prepared by the methylation of α-amino-β-hydroxy-acids. The betaïnes are widely distributed in plants, probably because of this relationship to the proteins; this is borne out by the fact that betaïnes occur most abundantly in those parts of the plant in which the vegetative processes are most active, for instance, in germinating seeds and in young leaves. Young Sugar Beet leaves contain as much as 2·5 per cent. betaïne, while older ones contain about 1 per cent. Similarly the content of *stachydrine* in Orange leaves decreases with increasing age of the tissue. Again, injection of the stems of Rice with proline, ornithine, or glutamic acid (all amino-acids) increases the yield of *trigonelline* in the plant juice. Betaïnes can act as methylating agents, and may do so in some plants, but in most cases they are probably by-products in protein katabolism.

Above 100° C., when they lose water of crystallisation, betaïnes behave as internal salts; $R\overset{+}{N}\cdot CH_2CO\overset{-}{O}$.

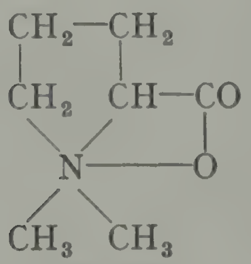
Betaïne itself, $C_5H_{11}O_2N$, is the anhydride of trimethylglycine (I). Below 100° C., it crystallises with one molecule of water, when it is probably represented by formula (II):


(I)

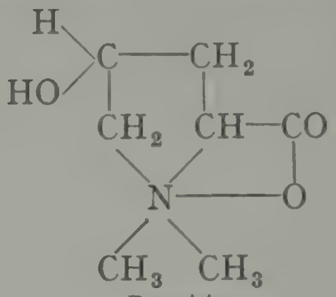

(II)

Betaïne occurs in a variety of plants. It is present in all species of *Chenopodiaceæ* examined, including the Sugar Beet (*Beta vulgaris*, hence the name betaïne), *Chenopodium vulvaria*, which, as has been noted already, gives off trimethylamine from the decomposition of betaïne, and *Atriplex canescens*, which contains 3·78 per cent. betaïne in the dried leaves. Betaïne also occurs in some of the *Solanaceæ*, *e.g. Lycium barbarum*, in which it was first discovered, and in many genera of the *Amarantaceæ*.

Stachydrine, $C_7H_{13}O_2N$, occurs in several of the *Labiatæ*, *viz.* in tubers of *Stachys tuberifera*, in *Betonica officinalis*, and in *Galeopsis ochroleuca*. It is also found in leaves of the Orange Tree (*Citrus Aurantium*), and in flowers of *Chrysanthemum cinerariæfolium*. In *Galeopsis* it is present in the *lævo*-form, in the others it is optically inactive. It is the anhydride of a methylated amino-acid, *proline*, and is therefore a true betaïne, although on account of its pyrrolidine ring (p. 222), it is often classed with the alkaloids:



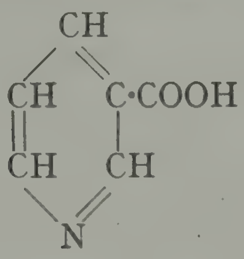
Stachydrine



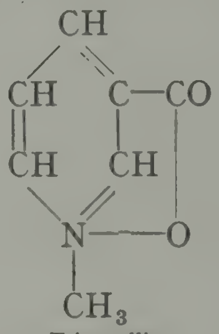
Betonicine

Betonicine and **Turicine,** $C_7H_{13}O_3N$, are *laevo-* and *dextro-*rotatory forms of the corresponding derivative of *oxy-proline*, and occur with stachydrine in the Betony.

Trigonelline, $C_7H_7O_2N$, is the betaïne derived from nicotinic acid. It was first discovered in the seeds of the Fenugreek (*Trigonella Fœnum-græcum*); it also occurs in seeds of the Pea, Kidney Bean, Oat and Hemp, in tubers of Potato, Dahlia, and *Stachys tuberifera*, and in roots of *Scorzonera hispanica*. Nicotinic acid contains the pyridine nucleus (p. 220), and is related to the alkaloids. Its acid amide, **nicotinamide,** $C_5H_4N{\cdot}CONH_2$, is one of the components of the vitamin B complex, and is part of several respiratory enzymes (p. 170). Amino-acids are also the source of nicotinic acid in the plant.



Nicotinic acid

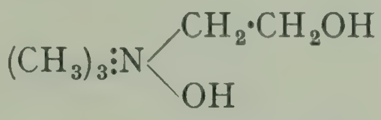


Trigonelline

Choline and Colamine

Choline and colamine are **strong bases of multiple function** and are components of the phospholipins, which occur in all living cells. Choline is the more common, being the base present in *lecithin* (p. 51); it also occurs to a small extent in the free state in many plants, especially in seeds, where it is formed by the hydrolysis of lecithin. For instance, during the germination of Vetch (*Vicia sativa*), the choline content rises from 0·017 per cent. in the seeds to 0·06 in four-week-old seedlings, owing to the hydrolysis of lecithin, which shows a parallel decrease from 0·74 to 0·19 per cent. Other plant tissues in which choline has been found are seeds of Pea, Bean, Lentil, Oat, Hemp, Cotton; seedlings of Lupin, Soya Bean, Rice, Barley, Wheat, and Vegetable Marrow; Orange leaves and the green parts of Meadow Sage (*Salvia pratensis*) and Betony; flowers of *Chrysanthemum cinerariæfolium*; fruit of Hops and Grapes and several Nuts (*e.g. Areca* and *Arachis hypogæa*); tubers of Potato, Dahlia, and *Stachys tuberifera*; roots of Beet, Henbane (*Hyoscyamus*), and Deadly Nightshade (*Atropa Belladonna*); and the underground stems and roots of Cabbage, Celery, Jerusalem Artichoke, Carrot, Chicory, and *Scorzonera.*

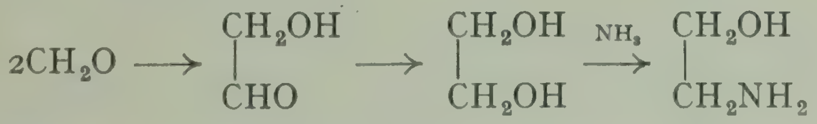
Choline on isolation is obtained as a hygroscopic, crystalline mass with a strongly basic reaction. It is trimethyl-β-hydroxyethyl-ammonium hydroxide:



$$(CH_3)_3{:}N\begin{array}{l} \diagup CH_2\cdot CH_2OH \\ \diagdown OH \end{array}$$

It is the corresponding alcohol to betaïne, which contains the acidic group, and choline can be oxidised in the laboratory to betaïne. This relationship may have fundamental importance in the synthesis of the phospholipins, although the origin of free betaïne in plants is usually protein, and of choline the phospholipins. There is, however, a possibility that betaïne replaces choline in some of the phospholipins. In the animal system, choline appears to be one of the B-complex vitamins necessary for nutrition. It acts as a methylating agent.

Colamine, or amino-ethyl alcohol, $CH_2(NH_2)\cdot CH_2OH$, occurs in various plant phospholipins, for example, in those of Beans, Peas, and Oats. It may also be a precursor of choline, which would be formed by methylation of colamine, and it is probably synthesised *de novo* in the plant. Trier suggests that it is formed by the action of ammonia on glycol, which in turn could be derived from formaldehyde through glycollic aldehyde:



$$2CH_2O \longrightarrow \begin{array}{l} CH_2OH \\ | \\ CHO \end{array} \longrightarrow \begin{array}{l} CH_2OH \\ | \\ CH_2OH \end{array} \xrightarrow{NH_3} \begin{array}{l} CH_2OH \\ | \\ CH_2NH_2 \end{array}$$

Muscarine is a very poisonous base occurring in the Fly Agaric (*Amanita muscaria*) and is related to both choline and betaïne. It is probably a by-product in protein metabolism in plants, and not directly connected with the phospholipins.

CHAPTER XIV

AMINO-ACIDS AND AMIDES

Amino-acids

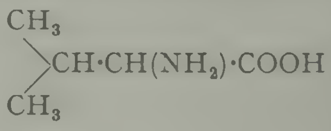
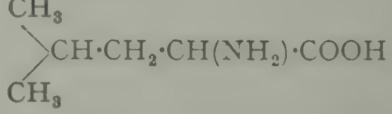
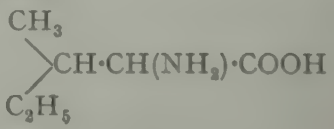
Amino-acids are carboxylic acids in which an *amino* group replaces a hydrogen atom on one of the carbon atoms in the molecule. The most important amino-acids are those which form the building-stones of the protein molecule, and are obtained from proteins by hydrolysis. Nineteen such acids which have been obtained from plant protein are listed in the accompanying table (IV). In all cases except two, *viz.* proline and oxyproline, the amino group is *primary* (—NH_2), and is attached to the carbon atom next to the carboxyl group, *i.e.* they are α-amino-acids. The acids may be grouped as derivatives of the *fatty acids, e.g.* glycine, $CH_2(NH_2)\cdot COOH$ (derived from acetic acid), of the *dibasic acids, e.g.* aspartic acid (derived from succinic acid), and of acids containing ring structures. These cyclic structures may be either the *aromatic benzene ring,* as in phenylalanine and tyrosine, or *heterocyclic* rings such as the *pyrrolidine ring* (p. 222) in proline and oxyproline, the *iminazole* ring (p. 162) in histidine, and the *indole* ring (p. 226) in tryptophan.

Table IV

Amino-Acids from Plant Proteins

Aliphatic

Monocarboxylic monoamino acids :

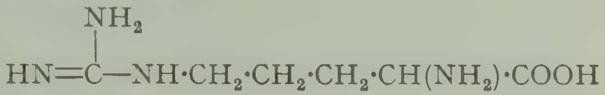
Glycine: α-amino-acetic acid.	$CH_2(NH_2)\cdot COOH$
Alanine: α-amino-propionic acid.	$CH_3\cdot CH(NH_2)\cdot COOH$
Serine: α-amino-β-hydroxy-propionic acid.	$CH_2OH\cdot CH(NH_2)\cdot COOH$
Threonine: α-amino-β-hydroxy-butyric acid.	$CH_3\cdot CHOH\cdot CH(NH_2)\cdot COOH$
Valine: α-amino-*iso*-valeric acid.	 $\left.\begin{matrix}CH_3\\ CH_3\end{matrix}\right\rangle CH\cdot CH(NH_2)\cdot COOH$
Leucine: α-amino-*iso*-caproic acid.	 $\left.\begin{matrix}CH_3\\ CH_3\end{matrix}\right\rangle CH\cdot CH_2\cdot CH(NH_2)\cdot COOH$
*iso*Leucine: α-amino-β-methyl-β-ethyl-propionic acid.	 $\left.\begin{matrix}CH_3\\ C_2H_5\end{matrix}\right\rangle CH\cdot CH(NH_2)\cdot COOH$

Dicarboxylic monoamino acids :

Aspartic acid: α-amino-succinic acid. $COOH\cdot CH_2\cdot CH(NH_2)\cdot COOH$
Glutamic acid: α-amino-glutaric acid. $COOH\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$

Monocarboxylic diamino acids :

Arginine: δ-guanidine-α-amino-valeric acid.

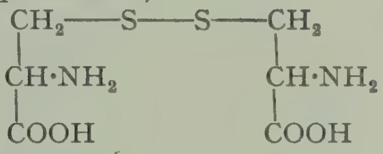


$$HN{=}\overset{\overset{NH_2}{|}}{C}{-}NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$$

Lysine: αε-diamino-caproic acid:
$CH_2(NH_2)\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$

Sulphur-containing amino acids :

Cystine or dicysteïne: di-(β-thio-α-amino-propionic acid).

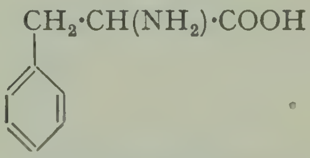


Methionine: α-amino-γ-methylthiol-*n*-butyric acid.
$CH_3\cdot S\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$

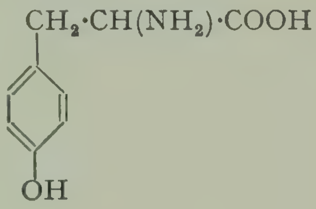
Aromatic

Monocarboxylic monoamino acids :

Phenylalanine: β-phenyl-α-amino-propionic acid.

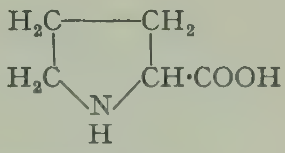


Tyrosine : *p*-hydroxy-phenylalanine.

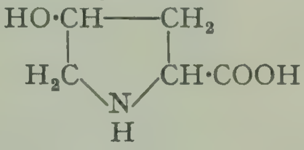


Heterocyclic

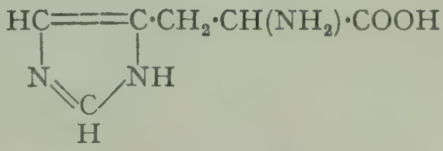
Proline: α-pyrrolidine-carboxylic acid.



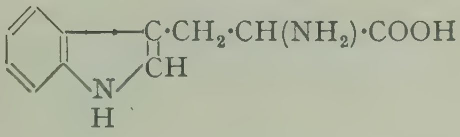
Hydroxyproline (Oxyproline): γ-hydroxy-α-pyrrolidine-carboxylic acid.



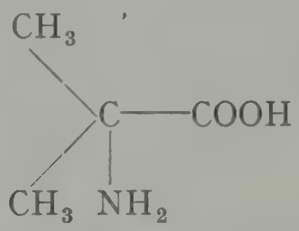
Histidine: β-iminazole-alanine.



Tryptophan: β-indole-alanine.

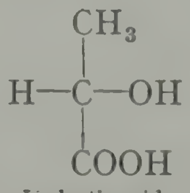


There are a few similar amino-acids which may also be constituents of the protein molecule; but claims for their presence as original units in the protein molecule, and not simply as secondary products of protein decomposition, have not yet been sufficiently substantiated to justify their inclusion in the table. α-Amino-*iso*-butyric acid has been isolated from the protein of Lupin seeds by enzymatic decomposition. This appears to constitute the first case of the occurrence of a protein amino-acid having the amino group attached to a tertiary carbon atom:



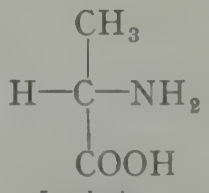
Hydroxy-lysine, $CH_2(NH_2)\cdot CH_2\cdot CH_2\cdot CH(OH)\cdot CH(NH_2)\cdot COOH$, has also been isolated from vegetable proteins, *viz.* the proteins from Oats, Cabbage leaves, and Hemp seed.

Physical Properties of the Amino-acids. The amino-acids are all white, crystalline substances, soluble in water. The least soluble are tyrosine and cystine, which may therefore be isolated by crystallisation from concentrated solutions. They are all readily diffusible; hence they are formed by the hydrolysis of reserve protein during the germination of seeds, so that soluble nitrogenous compounds can be translocated to the growing areas of plumule and radicle. With the exception of proline, the amino-acids are all precipitated by ethyl alcohol, but are not precipitated by saturation of the solution with ammonium sulphate or sodium chloride; this behaviour serves to distinguish them from the soluble proteins. The mono-amino-acids are soluble also in butyl alcohol, and this property forms the basis of Dakin's method for the separation of the amino-acids obtained by the hydrolysis of proteins. Many of the amino-acids, *e.g.* glycine, have a sweet taste, but *iso*leucine and arginine are bitter. As in the sugars (p. 65), spatial isomerism due to the different arrangement of the groups on the α-carbon atom is possible. Lactic acid is taken as the conventional reference substance, and naturally occurring amino-acids all belong to the *L*-series:



$$\begin{array}{c} CH_3 \\ | \\ H-C-OH \\ | \\ COOH \end{array}$$

L—lactic acid



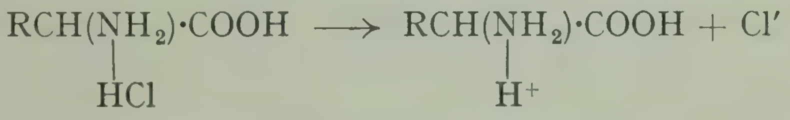
$$\begin{array}{c} CH_3 \\ | \\ H-C-NH_2 \\ | \\ COOH \end{array}$$

L—alanine

Asymmetric carbon atoms are present in all amino-acids except glycine, and in nature only one optically active form of each amino-acid is found, some being *dextro-* and some *laevo*-rotatory. The rotation is indicated by the signs (+) or (−), as in the sugars. Naturally occurring alanine is $L(+)$-alanine.

Chemical Properties. By virtue of the basic amino group, all the amino-acids can form salts with acids, both inorganic and organic. Similarly, the carboxyl group permits them to form salts with bases. They are therefore soluble both in dilute acids and alkalis (except cystine and arginine which are decomposed by alkali), and many of them form crystalline salts with metallic bases and with mineral acids. For instance, they form copper salts when an aqueous solution of the amino-acid is boiled with copper oxide, hydroxide, carbonate, or acetate, and the silver salts are used to isolate arginine and histidine from mixtures. Also some form salts with phosphotungstic acid, and this property is used in the isolation of lysine, arginine, and histidine.

Since amino-acids act both as acids and bases, their salts with mineral acids, *e.g.* hydrochloric acid, ionise in solution so that the amino-acid furnishes the *cation* and the mineral acid the anion (I); on the other hand, their salts with strong bases, *e.g.* sodium hydroxide, ionise so that the amino-acid furnishes the *anion* and the metal the cation (II):

(I) 

$$\text{RCH}(\underset{\text{HCl}}{\underset{|}{\text{NH}_2}})\cdot\text{COOH} \longrightarrow \text{RCH}(\underset{\text{H}^+}{\underset{|}{\text{NH}_2}})\cdot\text{COOH} + \text{Cl}'$$

$$\text{(II)}\quad \text{RCH}(\text{NH}_2)\cdot\text{COONa} \longrightarrow \text{RCH}(\text{NH}_2)\cdot\text{COO}' + \text{Na}^+$$

If an electric current is passed through the first solution, the amino-acid ion will move to the cathode; while in the second, it will move to the anode. Substances which can ionise both as anions and cations are called **amphoteric electrolytes.** At a definite p_{H} value for each amino-acid, there will be as many anions as cations in the solution, and this is called the **isoelectric point.** At this point there is no movement of amino-acid on electrolysis of the solution. The *proteins*, like the amino-acids, are amphoteric electrolytes.

Those amino-acids which contain only one amino group and one carboxyl group are neutral in aqueous solution. Three important amino-acids contain more than one carboxyl group, and are therefore acidic, *viz.* aspartic, glutamic, and hydroxyglutamic acids. Three others contain more than one basic group, and are therefore basic, *viz.* arginine and lysine, which contain two amino groups, and histidine, which contains one primary amino group and the basic iminazole ring.

Solutions of the amino-acids, by virtue of the primary amino group, evolve nitrogen on treatment with nitrous acid (p. 125), and this reaction has been utilised by van Slyke in the estimation of individual amino-acids and of their mixtures obtained in the hydrolysis of proteins (**Amino Nitrogen**). Half the nitrogen collected is derived from the amino-acid molecule:

$$R{\cdot}CH(NH_2){\cdot}COOH + HNO_2 \longrightarrow R{\cdot}CH(OH){\cdot}COOH + N_2 + H_2O$$

Another method of estimating amino-acids was devised by Sörensen. If an amino-acid is treated with formaldehyde, condensation takes place with the amino group, leading to a *methylene* derivative. This blocks the amino group, and the compound can then be titrated with alkali as a carboxylic acid:

$$H{\cdot}CHO + H_2N{\cdot}CH_2{\cdot}COOH \longrightarrow H_2C{=}N{\cdot}CH_2{\cdot}COOH + H_2O$$

A third method for the estimation, and separation, of various amino-acids obtained on hydrolysis of protein is due to Engeland. The amino-acids are exhaustively methylated to compounds of the betaïne type, which are easily precipitated and characterised by means of their double salts with the chlorides of the heavy metals, mercury, platinum, and gold.

EXPT. 52. *Reactions of Glycine*

1. Show that glycine contains nitrogen by the soda-lime test.
2. Show that glycine dissolves in water, giving a neutral solution. Divide this in two for the remaining tests.
3. Add copper sulphate solution to the glycine solution; a deep blue colour is obtained.
4. Add dilute hydrochloric acid to the glycine solution, then sodium nitrite solution; nitrogen is evolved with effervescence.

The reactions of the amino-acids are of importance in the study of nitrogen metabolism in plants, in animals, and in the soil. In plants, the amino-acids are formed by the hydrolysis of protein, especially during the germination of seeds, but they may also be synthesised *de novo* in the green plant. They are to some extent translocated as such to other tissues in the plant, but they may also be converted into other soluble compounds, *e.g.* amides, and also into non-nitrogenous compounds. Amino-acids can be **deaminated**—that is, decomposed with the loss of ammonia—by hydrolytic, oxidative, and reductive reactions, as is indicated by the following equations:—

(i) $R{\cdot}CH(NH_2){\cdot}COOH + H_2O \longrightarrow NH_3 + R{\cdot}CH(OH){\cdot}COOH$
α-hydroxy acid

(ii) $R{\cdot}CH(NH_2){\cdot}COOH + O \longrightarrow NH_3 + R{\cdot}CO{\cdot}COOH$
α-keto-acid

(iii) $R{\cdot}CH(NH_2){\cdot}COOH + O_2 \longrightarrow NH_3 + CO_2 + R{\cdot}COOH$
fatty acid

(iv) $R{\cdot}CH(NH_2){\cdot}COOH + H_2 \longrightarrow NH_3 + R{\cdot}CH_2{\cdot}COOH$
fatty acid

According to equation (i), glycine and aspartic acid would give glycolic and malic acids respectively, both of which are found in plants. It has been mentioned already (p. 122) that the source of acids in certain plants, such as Rhubarb and Begonia, is not carbohydrate as in most plants, but amino-acids which have undergone deamination, malic acid being the first product, while other acids, such as oxalic acid, are derived from malic acid by oxidation.

Several of the simpler acids, derivable according to equations (iii) and (iv) from amino-acids, are widely distributed in plants, especially succinic and glutaric acids, corresponding to aspartic and glutamic acids respectively.

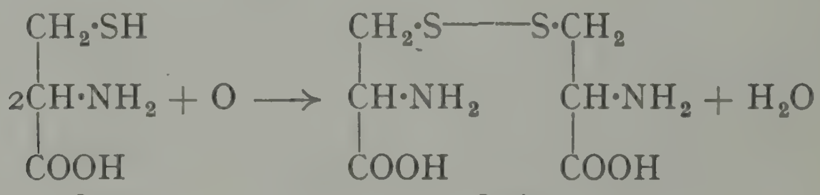
The reverse processes also take place in the plant, namely the action of ammonia on α-hydroxy- and α-keto-acids produced in respiration; this reversible process is called **transamination** and an enzyme **transaminase** is responsible *in vivo* (p. 290).

Free amino-acids occur in the higher plants mainly in the germinating seed, but small amounts of them are also found in other tissues. Almost all the amino-acids obtainable from plant proteins have also been isolated in the uncombined state from plants. Seedlings of Lupin (*Lupinus*), Vetch (*Vicia sativa*), and plants of Alfalfa (*Medicago sativa*) contain most of the amino-acids, but the same species sometimes shows differences in the amino-acids present, depending upon whether the seedlings are germinated in light or darkness.

Tyrosine is readily detected because of its oxidation by an enzyme **tyrosinase** (p. 261) to a black substance, *melanin*. It has been found in seedlings of Lupin, Pea, Vetch, Castor Bean (*Ricinus communis*), Vegetable Marrow (*Cucurbita Pepo*); in roots of Beet, and Cabbage (*Brassica oleracea*); in tubers of Potato, and Dahlia (*Dahlia variabilis*); in pods of Bean (*Phaseolus vulgaris*); and in the Alfalfa plant.

Tryptophan is also readily detected, as in faintly alkaline solution it gives a violet colour with bromine or chlorine water, and the colour can be extracted and stabilised with amyl alcohol. Tryptophan does not give this reaction when bound in the protein molecule, and hence this test is used to follow the course of hydrolysis of proteins containing tryptophan. It has been detected in seedlings of Lupin, Pea, Vetch, and Soya Bean (*Glycine hispida*).

Cystine occurs only in small amounts in plant proteins and is therefore hard to detect in the free state. It was thought for a long time that cystine was the only amino-acid containing sulphur. This element can be tested for by boiling the amino-acid or protein with strong sodium hydroxide solution, and then adding lead acetate solution. A black precipitate of lead sulphide is developed when sulphur is present. Cystine is built up from two molecules of *cysteïne*—which contains the sulphydryl group (—SH)—by the removal of two atoms of hydrogen. This is of special importance in glutathione (p. 157), which contains cysteïne in the molecule, and undergoes a similar oxidation:

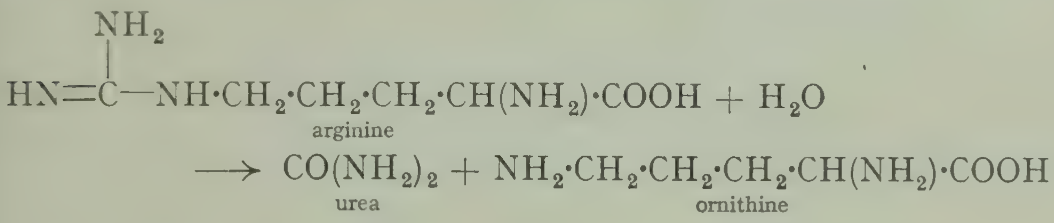


$$2\begin{array}{l}CH_2{\cdot}SH\\ |\\ CH{\cdot}NH_2\\ |\\ COOH\end{array} + O \longrightarrow \begin{array}{lcl}CH_2{\cdot}S & \text{———} & S{\cdot}CH_2\\ | & & |\\ CH{\cdot}NH_2 & & CH{\cdot}NH_2\\ | & & |\\ COOH & & COOH\end{array} + H_2O$$

Cysteïne Cystine

Methionine is another sulphur-containing amino-acid which has been discovered as a constituent of both plant and animal proteins. It has been obtained among the hydrolytic products of *edestin*, the protein of Hemp seed (*Cannabis sativa*), and of the *gluten* of Maize (*Zea Mays*). Its structure has been proved by synthesis. *In vivo* it acts as a methylating agent.

Arginine is widely distributed in seeds and seedlings, especially of the *Leguminosæ* and the *Coniferæ*. It also occurs in roots and tubers, including those of the Beet, Potato, Dahlia, Chicory, Artichoke, and Turnip. An enzyme which decomposes arginine by the hydrolysis of the molecule at a C—N linkage, giving another amino-acid, **ornithine,** and urea, also occurs in plants and is called **arginase.** According to Krebs, urea is formed in the animal organism through the intermediary of ornithine; this substance reacts with ammonia and carbon dioxide to form arginine, which in turn breaks down into ornithine and urea, through the action of arginase of the liver, thus giving rise to a never-ending cycle. Ornithine does not accumulate in the plant, but traces of urea have been found, and a widely distributed enzyme called **urease** decomposes urea still further to ammonium carbonate. Ornithine can, however, be obtained *in vitro* by the action on arginine of arginase extracts from Wheat and Vetch seedlings. Since these seedlings also contain urease, urea does not accumulate:



$$\underset{\text{arginine}}{HN{=}\overset{\overset{NH_2}{|}}{C}{-}NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH} + H_2O \longrightarrow \underset{\text{urea}}{CO(NH_2)_2} + \underset{\text{ornithine}}{NH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH}$$

3, 4-**Dihydroxyphenylalanine** is an amino-acid which, although it has not been detected as a constituent of either plant or animal protein, occurs free in all parts of the Broad Bean (*Vicia Faba*) and also in the Velvet Bean (*Stizolbium*). It is an oxidation product of tyrosine, and with the enzyme tyrosinase forms an oxidising system in plant respiration (p. 262). It is readily oxidised in air to give a black pigment, which appears on the destruction of the tissues of the Broad Bean, and is probably also the cause of the black spots on the flower.

Acid Amides

Acid amides, or amides, are derivatives of carboxylic acids, in which the primary amino group replaces the —OH of the carboxyl group. The general formula is therefore $R\cdot CO\cdot NH_2$. The simple amides are usually crystalline solids, soluble in water giving neutral solutions. They are decomposed on being warmed with dilute alkalis to give ammonia and the salt of the corresponding acid (*a*); boiling with mineral acids gives the free carboxylic acid and the ammonium salt of the mineral acid (*b*):

$$(a)\quad R\cdot CO\cdot NH_2 + NaOH \longrightarrow R\cdot COONa + NH_3$$

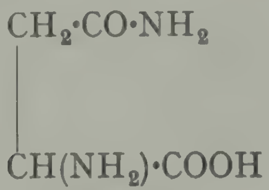
$$(b)\quad R\cdot CO\cdot NH_2 + HCl + H_2O \longrightarrow R\cdot COOH + NH_4Cl$$

Nitrous acid also reacts with amides to form the free acid with evolution of nitrogen:

$$R\cdot CO\cdot NH_2 + HNO_2 \longrightarrow R\cdot COOH + N_2 + H_2O$$

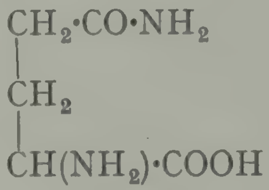
The acid amide grouping also occurs in proteins, hence the hydrolysis of these substances with acid (equation (*b*)) leads to the formation of ammonium salts in solution. On distillation of the solution with lime or magnesia, preferably *in vacuo* to prevent the decomposition of cystine and arginine, the ammonia is evolved, and collected in standard acid. This gives a measure of the **Amide Nitrogen,** as distinct from other forms of nitrogen, such as amino nitrogen, in the protein molecule.

The most important plant amides are amides of the dicarboxylic amino-acids, aspartic and glutamic acids, *viz.* **asparagine** and **glutamine** respectively:



$$\begin{array}{l} CH_2{\cdot}CO{\cdot}NH_2 \\ | \\ | \\ CH(NH_2){\cdot}COOH \end{array}$$

Asparagine



$$\begin{array}{l} CH_2{\cdot}CO{\cdot}NH_2 \\ | \\ CH_2 \\ | \\ CH(NH_2){\cdot}COOH \end{array}$$

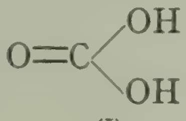
Glutamine

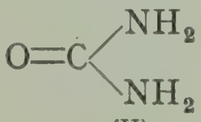
Asparagine and glutamine are very widely distributed in plant tissues, appearing instead of aspartic and glutamic acids on the hydrolysis of protein, especially in germinating seedlings. The discovery that they are not derived *only* from these acids marked an important step in the investigation of nitrogen metabolism in plants (p. 293). Asparagine and glutamine units may also be present in protein molecules, as they have been obtained by enzymatic hydrolysis of edestin and gliadin.

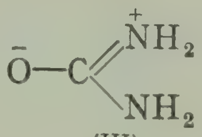
Asparagine has been found in all the *Leguminosæ* and *Gramineæ* examined, also in many other seeds and seedlings, *e.g.* Fir, Pine, Nasturtium, Sunflower, Poppy; in roots or tubers of Beet, Cabbage, Dahlia, Potato, Jerusalem Artichoke, *Scorzonera*; and in buds of Horse Chestnut, Plane, Sycamore, Poplar, Beech, Lime, and Alder. It can be prepared from Asparagus shoots (*Asparagus officinalis*) by precipitation with mercuric nitrate from the aqueous plant extract, after removal of protein. The precipitate is decomposed with sulphuretted hydrogen, and the asparagine isolated from solution by precipitation with alcohol, when it is obtained in the form of small colourless crystals.

Glutamine also is widely distributed, especially in seedlings of the *Cruciferæ* and *Caryophyllaceæ.* It has also been isolated from roots of Beet, Carrot, and Radish.

Urea, the most important form of waste nitrogen excreted by the animal organism, is also found in small amounts in a variety of plants, *viz.* Spinach (*Spinacia oleracea*), Cabbage, Potato, Carrot, Chicory, Soya Bean, and Wheat. Urea may be a universally distributed metabolic substance in plant tissues, as its existence would afford an explanation of the mode of synthesis of the purines and related substances. Its formation in the plant may be due entirely to the decomposition of arginine from the proteins (p. 137), but it may also be synthesised by urease (*vide infra*). Urea is a colourless, crystalline solid, m.p. 132° C. It is soluble in water and alcohol, and in the former gives a neutral solution. It does, however, contain a basic group, as it forms salts, *e.g.* urea nitrate, with strong acids. It may be regarded as a diamide (II) of carbonic acid (I), but some of its reactions can only be explained on the basis of the internal salt formula (III):


(I)


(II)


(III)

For instance, urea reacts with nitrous acid only in the presence of a mineral acid (*e.g.* if sodium nitrite and hydrochloric acid are used) to give nitrogen, carbon dioxide, and water:

$$CON_2H_4 + 2HNO_2 \longrightarrow 3H_2O + 2N_2 + CO_2$$

On heating urea above its melting-point, ammonia is evolved, and several substances are formed, including ammonium cyanate, $(NH_4)CNO$, cyanuric acid, $(CHON)_3$, and biuret:

$$NH_2{\cdot}CO{\cdot}\boxed{NH_2 + H}NH{\cdot}CO{\cdot}NH_2 \longrightarrow NH_3 + \underset{\text{biuret}}{NH_2{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2}$$

If the residue is shaken with water, to which a few drops of sodium hydroxide and copper sulphate solutions are then added, a violet colour is developed, due to biuret. This colour test, known as the *biuret reaction*, depends on the repetition of the —CONH— grouping and is therefore given not only by biuret but by the *proteins* and other compounds which contain the same grouping.

Urea is hydrolysed when warmed with dilute aqueous alkali, ammonia being liberated:

$$CON_2H_4 + 2NaOH \longrightarrow Na_2CO_3 + 2NH_3$$

The enzyme **urease,** which is very widely distributed in plants, decomposes urea into ammonia and carbon dioxide. It occurs especially in the *Leguminosæ*, being present in relatively large amounts in the Soya Bean (*Glycine hispida*), the Sword Bean (*Canavalia gladiata*), and the Jack Bean (*Canavalia ensiformis*); it is found in smaller amounts in species of *Lupinus*, *Vicia*, *Cytisus*, *Medicago*, *Pisum*, and *Trifolium*. Urease occurs also in Wheat seedlings, together with arginase. Sumner showed that the enzyme first hydrolyses urea to ammonium carbamate, $CO(NH_2)(ONH_4)$, which is then hydrolysed further to ammonium carbonate,

$(NH_4)_2CO_3$. Urease can also catalyse the formation of urea from ammonium carbonate *in vitro*, and this synthesis of urea may take place in the plant.

Acid amides structurally derived by the condensation of an acid with an amine are also possible, and have the general formula, $R{\cdot}CO{\cdot}NH{\cdot}R'$. Several such substances occur as the pungent principle of certain plants, *e.g.* **pellitorine** from *Anacyclus pyrethrum* and **capsaicin** from the Red Pepper (*Capsicum annuum*). In these cases, R represents unsaturated aliphatic acids, *e.g.* $C_9H_{17}COOH$ in capsaicin, while R′ is either an aliphatic or aromatic amine, *e.g.* *iso*butylamine in pellitorine and vanillyl-amine (compare vanillin, p. 183), $C_6H_3(OH)(OCH_3)CH_2NH_2$, in capsaicin.

CHAPTER XV

PROTEINS

PROTEINS are *laevo*-rotatory compounds of high molecular weight, containing C, H, O, N, and S, and in some cases phosphorus. The carbon content varies from 50–55 per cent., hydrogen from 6·5–7·3, oxygen from 20–24, nitrogen from 15–19, and sulphur from 0·3–5.

Occurrence. Proteins are found in the living parts of all plants, and it was from their occurrence in all cell protoplasm, both plant and animal, that they derived their name meaning 'to be first.' They occur in various states in plants: they are present *in colloidal solution* in the cell-sap; in the *semi-solid state*, as a very viscid colloidal sol in the protoplasm; and also in the *solid form* as reserve protein in seeds, roots, tubers, and bulbs. This solid protein sometimes occurs as well-developed crystals both in seeds and in underground tissues (being suspended in the latter case in the cell-sap), or it may occur in those semi-crystalline or amorphous bodies, the '*aleurone grains*,' in seeds, especially *Ricinus*. Seeds are the most concentrated source of plant protein, and therefore the reserve proteins of seeds have been the most widely studied. In monocotyledons, such protein is practically all in the endosperm, *e.g.* in cereals; whereas in dicotyledons, *e.g.* in legumes, the cells containing the reserve protein are partly in the endosperm and partly in the embryo. Cell nuclei, and therefore also embryos, contain protein; some of this is in a more complex form, as it is combined with other substances, especially nucleic acids, forming the **nucleoproteins.** In the process of milling, Wheat embryos are separated from the endosperms, and have provided a source of such proteins for investigation. Chibnall and his co-workers have also separated the cell-sap from the cell cytoplasm in leaves of various plants, and have isolated and examined the proteins from these two sources. All plant *viruses* which have been investigated chemically are nucleoproteins; animal viruses are more complex, as they appear to contain phospholipins as well. Many *enzymes* are conjugated proteins; in the protein part of the complex lies the specificity of the enzyme, while the chemical reaction (reduction, etc.) is accomplished by the attached, or *prosthetic* group.

Isolation. Proteins are isolated from plants (i) by extraction with various solvents, followed by precipitation from the resulting solutions, often with inorganic salts such as ammonium sulphate or sodium sulphate, (ii) by dilution, or (iii) by dialysis. As their chemical structure has not been established to such a degree as will enable it to serve as a basis of classification, differences in their solubilities are the deciding factor in the classification of the simple proteins. This distinction in solubility is, however, borne out chemically by the relative amounts of amino-acids obtained by hydrolysis of the different groups of proteins, and botanically by their distribution in seeds of different natural orders.

Colour Reactions. The presence of protein in plant tissues or extracts can be shown by several colour tests, most of which (*e.g.* Millon's and Xanthoproteic tests) are due to some specific amino-acid or amino-acid grouping in the molecule, the exception being the biuret reaction, which depends on the constitution of the protein molecule.

(1) The **Xanthoproteic test,** in which protein develops a yellow colour with concentrated nitric acid, is due to the formation of a yellow nitro-derivative of an aromatic nucleus, and therefore to the presence of the amino-acids tyrosine and tryptophan in the molecule.

(2) **Millon's Reaction,** in which the protein is warmed with mercurous nitrate in nitric acid (Millon's reagent), gives a red precipitate or solution due to the presence of a phenolic grouping in the molecule (which occurs in tyrosine).

(3) The **Biuret Reaction,** in which the protein gives a violet colour with sodium hydroxide and a little copper sulphate solution, is characteristic of a repetition of the grouping —CONH— (p. 139). All proteins do not give all the colour tests; for instance, gelatine contains no tyrosine and therefore does not give Millon's test.

EXPT. 53. *Colour Tests for Proteins*

(i) Xanthoproteic Test. Add concentrated nitric acid to a protein or its solution and warm. A yellow colour or precipitate is obtained, which turns orange on adding ammonium hydroxide to the cooled solution till alkaline.

(ii). Millon's Test. [Prepare Millon's reagent by dissolving gradually 15 c.c. of mercury in 285 c.c. of concentrated nitric acid in a beaker in a fume chamber. Stir occasionally until brown fumes are no longer evolved. Dilute the resulting solution with twice its volume of water.]

Warm a little of the protein or its solution with Millon's reagent, when a pink or red colour or precipitate is developed.

(iii) Biuret Test. To a protein solution add sodium hydroxide solution, then *one drop* of 2·5 per cent. copper sulphate solution, when a violet colour is produced (p. 139).

Classification. Plant Proteins are grouped according to the following scheme :—

I. Simple proteins
- (*a*) Albumins
- (*b*) Globulins
- (*c*) Prolamins
- (*d*) Glutelins
- (*e*) Histones
- (*f*) Protamins

II. Conjugated proteins
- (*a*) Metalloproteins
- (*b*) Hæmoproteins
- (*c*) Flavoproteins
- (*d*) Nucleoproteins
- (*e*) Nucleotide Proteins
- (*f*) Thiamine Proteins
- (*g*) Lipoproteins

III. Derived proteins
- (i) Primary Derivatives:
  - (*a*) Proteans
  - (*b*) Metaproteins
  - (*c*) Coagulated Proteins
- ii) Secondary Derivatives:
  - (*a*) Proteoses
  - (*b*) Peptones
  - (*c*) Peptides

The group of derived proteins includes all substances obtained by a modification of the protein molecule by such reagents as heat, acid and alkali, and enzymes. These will be dealt with later.

Of the simple proteins, prolamins and glutelins are exclusively plant proteins, and have not been found in the animal kingdom; they are peculiarly the proteins of cereal grain (*Gramineæ*). The globulins are the most widely distributed in plants, occurring as reserve protein especially in the *Leguminosæ* and in fat-containing seeds. In general it is found that similar or identical proteins are found only in seeds of the same natural order; for instance, the globulins of the *Leguminosæ* are very similar among themselves, but differ appreciably from the globulins of non-leguminous seeds.

The proteins in different tissues of the same plant may be different, especially if they have a different function in the plant metabolism. The following table shows such a difference between the types of protein in Wheat embryos and the whole kernel. Much of the globulin and proteose in the whole kernel, and some of the albumin, undoubtedly come from the embryo and not the endosperm:—

	Whole wheat kernel (per cent.)	Wheat embryo per cent.)
Albumin	0·4	10
Globulin	0·6	5
Prolamin	4·0	0
Glutelin	4·7	0
Proteose	0·2	3

SIMPLE PROTEINS

(*a*) **Albumins.** Albumins, which derive their name from egg albumin, are characterised by their solubility in water. They are all coagulated by heat. Plant albumins, unlike animal albumins, can be precipitated from neutral solution with sodium chloride or magnesium sulphate. Albumins occur in plants in relatively small amounts (up to about 3 per cent. of the dry weight), but they are fairly widely distributed in plant juices, and in seeds, where they are associated with other proteins. The best-defined albumins are:

Leucosin in Wheat (*Tricitum vulgare*), Barley (*Hordeum vulgare*), and Rye (*Secale cereale*).

Legumelin in the Pea (*Pisum sativum*), Broad or Horse Bean (*Vicia Faba*), Vetch (*Vicia sativa*), Soya Bean (*Glycine hispida*), Lentil (*Ervum lens*), and some other legumes.

Phaselin in the Kidney or Navy Bean (*Phaseolus vulgaris*).

Ricin in the Castor Bean (*Ricinus communis*). This is very toxic and therefore is often referred to as a toxalbumin.

Another toxalbumin has been found in the bark of the False Acacia (*Robinia pseud-acacia*).

Leucosin of Wheat occurs definitely in the embryo, and not in the endosperm of the grain; and probably leucosin of the other cereals, and legumelin, are also obtained from the physiologically active tissues, rather than from the store of reserve protein.

EXPT. 54. *Albumins*

1. Take the expressed juice of mangolds (preferably those which have been stored for some time) and perform the following tests on separate portions in test-tubes:—

(*a*) Show that the protein present coagulates on boiling.

(*b*), (*c*), and (*d*) Colour tests as on p. 142.

2. Steep 10 grm. portions of (i) wheat flour, (ii) pea flour, (iii) soya bean meal, in 100 c.c. of water for about 1 hour. Decant through filter-paper in filter-funnels and test the solutions for protein by tests (*a*) to (*d*) above.

(*e*) Show that in each case the protein is precipitated by saturation of the solutions with solid ammonium sulphate.

(*b*) **Globulins.** The globulins are defined as proteins insoluble in water but soluble in dilute salt solutions. As most plants contain a certain amount of inorganic salts, a water extract in some cases contains not only albumin but globulin (*e.g.* water extract of pea flour, above). The isolation of these proteins is also complicated by the fact that, like the amino-acids, they can form salts with acids, and these salts may have quite different solubilities from the free proteins. Legumin from some of the *Leguminosæ* and edestin from Hemp seed are the best examples of this; legumin itself is soluble in water, but as it is practically always present as the acid salt, it is only extracted by salt solutions. Edestin, on the other hand, is more soluble in dilute salt solutions than some of its salts. The plant globulins are not all coagulated on heating. They cannot all be precipitated from solution by saturation with magnesium sulphate, as are the animal globulins, but they are precipitated by sodium sulphate. Many of the globulins have been obtained in crystalline form, either by dilution of the warm salt solution (which is then allowed to cool), or by dialysis. A large number of plant globulins have been isolated and characterised, especially from the *Leguminosæ* and from fat-storing seeds. The following are representative globulins:—

From *Leguminosæ*:

Legumin from seeds of Pea, Broad Bean, Vetch, and Lentil.
Vicilin from Pea, Broad Bean, and Lentil.
Phaseolin from Kidney Bean.
Conglutin from Lupin (*Lupinus*).
Vignin from Cowpea (*Vigna sinensis* and *Vigna Catjang*).
Glycinin from Soya Bean.

From fat-containing seeds:

Excelsin from seeds of Brazil nut (*Bertholletia excelsa*).
Corylin from seeds of Hazel (*Corylus Avellana*).
Juglansin from European Walnut (*Juglans regia*), American Black Walnut (*Juglans nigra*), and American Butternut (*Juglans cinerea*).
Amandin from Almond (*Prunus Amygdalus*).
Castanin from Chestnut (*Castanea vulgaris*).
Arachin from Peanut (*Arachis hypogœa*).
Edestin from seeds of Hemp (*Cannabis sativa*).

From other seeds and plant tissues:

Avenalin from grain of Oat (*Avena sativa*).
Tuberin from tubers of Potato (*Solanum tuberosum*).

Small amounts of globulins are also found in many of the cereal grains (*Gramineæ*); much of this material is in the embryo, except in the case of the Oat, where some of it is reserve protein. Well-defined globulins to which distinctive names have not been given have been isolated from seeds of Flax (*Linum usitatissimum*), Squash (*Cucurbita maxima*), Sesame (*Sesamum indicum*), Cotton (*Gossypium herbaceum*), Radish (*Raphanus sativus*), Rape (*Brassica Napus*), Mustard (*Brassica alba*), Castor Bean (*Ricinus communis*), Coconut (*Cocos nucifera*), and Tomato (*Solanum Lycopersicum*).

EXPT. 55. *Globulins*

1. Take the residue from the extraction of pea flour and steep it with 100 c.c. of 10 per cent. sodium chloride solution for about an hour. Filter, and test the filtrate as above (*a*) to (*e*), p. 144.

2. Steep about 10 grm. of ground hazel nuts or almonds in 100 c.c. of 10 per cent. sodium chloride solution for an hour, filter, and test as above.

(*c*) **Prolamins.** These proteins derive their name from the large amounts of proline obtained from them on hydrolysis. Thus the cereals are related chemically by the nature of this group of proteins built up and stored by the grain. The prolamins are insoluble in water and dilute salt solution, but are soluble in 70–90 per cent. alcohol. The following prolamins have been characterised:—

Gliadin from Wheat and Rye. These gliadins appear to be identical.
Zein from Maize (*Zea Mays*).
Hordein from Barley (*Hordeum vulgare*).
A prolamin (unnamed) from Rice (*Oryza sativa*).

Some apparently simple proteins such as gliadin are not one substance but a mixture of proteins containing different percentages of the same amino-acid components. Changes in temperature, salt content, hydrogen ion concentration, etc., change the composition of the mixture (Sörensen).

(*d*) **Glutelins.** The glutelins, like the prolamins, are proteins of the cereals; but proteins isolated separately from the cell-sap (vacuoles) and from the cytoplasm of vegetative tissues of the higher plants have also been found to belong to this group. *Gluten* of Wheat is a mixture of *gliadin* (a prolamin) and *glutenin* (a glutelin). By fractional precipitation from solution with ammonium sulphate, this glutenin has been shown to consist of two glutelins, α and β. Glutelins are characterised by their insolubility in water, dilute salt solutions and alcohol, and by their solubility in dilute alkali and dilute acid.

The glutelins of seeds are:

Glutenin = α and β glutelins of Wheat.
Oryzenin of Rice.
Glutelins α and β of Maize.
Avenin of Oat.
Hordenin of Barley.
Unnamed glutelins of Cotton and Melon (*Cucumis Melo*).

Cell-sap glutelins have been isolated from the leaves of Spinach (*Spinacia oleracea*) and of Alfalfa (*Medicago sativa*); while cytoplasmic glutelins have been obtained from leaves of Spinach, Alfalfa, Maize, Cabbage, Horseradish (*Cochlearia Armoracia*), Sunflower (*Helianthus annuus*), and Fig (*Ficus Carica*).

Davies has investigated the protein of the combined expressed juice and water extracts of forage crops of the *Leguminosæ, viz.* Alfalfa (*Medicago sativa*), Sainfoin (*Onobrychis sativa*), Vetch (*Vicia sativa*), Broad Red Clover (*Trifolium pratense*), Crimson Clover (*Trifolium incarnatum*); of the *Cruciferæ, viz.* leaves of Cabbage, Marrow stem kale (*Brassica oleracea* var.), Kohlrabi (*B. oleracea* var.), White turnips (*Brassica napo-brassica*); and of the *Umbelliferæ*, *viz.* Carrot (*Daucus Carota*), and Parsnip (*Peucedanum sativum*). These proteins are probably mixtures and therefore difficult to classify according to the scheme for seed proteins; they are all coagulated by heat. The proteins from the *Cruciferæ* were comparable in percentages of the various amino-acids present with the globulin of Rape seed (*Brassica Napus*), except that the latter had a very high amide content.

Expt. 56. *Prolamins and Glutelins*

Wheat Gluten. Moisten 50 grm. of wheat flour with a little distilled water and make it into a stiff dough. Place this in a square of muslin and squeeze under running water till all the starch has been washed out. The residue in the muslin is a yellowish, sticky, elastic substance, gluten. Pull some of the gluten into small pieces and pound with rectified spirit. The stickiness disappears, as the gliadin dissolves, leaving the glutenin as an insoluble sediment. Decant the alcoholic solution (it is very difficult to filter), add more alcohol, and repeat the treatment.

1. Properties of Gliadin. Take some of the combined alcoholic extract and show that the protein is precipitated by water. Evaporate the remainder of the alcoholic extract to dryness on a water-bath, and perform tests (i) to (iii), p. 142, on the residue obtained.

2. Properties of Glutenin. With separate portions of the residue from the alcoholic extraction, show that glutenin is insoluble in water, and soluble in 0·5 per cent. sodium hydroxide solution; also carry out the colour tests (i) to (iii).

(*e*) and (*f*) **Histones** and **Protamins.** These proteins were originally isolated from fish sperm. They are strongly basic, as they contain a high proportion of the diamino-acids, especially arginine. They are differentiated by their solubility in water, protamins being soluble, whereas histones require dilute acid to dissolve them. It has been found that the proteins of the *nuclei* of both plant and animal cells contain a highly basic fraction, termed **nucleohistones** and **nucleoprotamins.** They are associated with the nucleic acid of the nucleus as **protein nucleates;** *i.e.* the linkage is salt-like, and therefore differs from the nucleoprotein discussed below. Fibrous nucleohistones have been isolated from Wheat germ, and contain a high percentage of tyrosine as well as arginine.

Animal proteins contribute an additional type of simple protein to the scheme on p. 143, *viz.* (*g*) scleroproteins (or albuminoids), of which the insoluble keratins of hair and horn are examples.

CONJUGATED PROTEINS

Conjugated proteins are complex compounds in which the protein molecule is combined or 'conjugated' with another molecule, called the *prosthetic group.* This group may be either inorganic, as in the copper proteins, organic as in the thiamine proteins, or contain both metallic and organic groups as in the hæmoproteins. In this last example, it is known that the metal is bound to the organic prosthetic group, but this is not always the case. The enzyme carboxylase requires magnesium for its activity, yet its prosthetic group is diphosphothiamine; both the magnesium and the thiamine are bound to the protein. Little is known about the structure of the protein moieties of the conjugated proteins. A considerable number of those which constitute enzyme systems appear to contain sulphydryl groups (p. 136) as a component essential for enzyme activity.

The first three groups are often called the **chromoproteins,** as their prosthetic groups are coloured.

(a) **Metalloproteins.** Several oxidising enzymes isolated from plants are protein which contain **copper** as an essential part of the molecule, *viz. tyrosinase* (*polyphenol oxidase*), *monophenol oxidase,* and *laccase.* They can all utilise atmospheric oxygen according to the equation: $XH_2 + \frac{1}{2}O_2 \longrightarrow X + H_2O$, that is, they are direct oxidases. Their function in the life processes of the plant is discussed later.

(b) **Hæmoproteins.** All living cells contain conjugated protein in which the prosthetic group is an **iron porphyrin.** The best known member is hæmoglobin, which can be split into the protein 'globin' and the prosthetic group 'hæmin'. A group of respiratory enzymes have been shown to contain similar iron-porphyrin compounds or hæmes as *coenzymes*, and these enzymes are universally distributed in plants as well as in animals. Through the organically bound iron they make oxygen transfer possible, and they are therefore oxidases (pp. 197 and 285).

Chlorophyll is a metallic porphyrin derivative, the metal being magnesium. Chlorophyll itself contains no protein, but in the chloroplasts chlorophyll is probably conjugated with protein. Very little chlorophyll can be removed from the cells until they are treated with alcohol. Again, the absorption bands of the mixed chloroplast pigments after isolation and of the chloroplasts themselves are different. Stoll suggested the term *chloroplastin* for the complex, and a substance of this type has been isolated. Hence another group containing chlorophyllprotein may parallel the hæmoproteins.

(c) **Flavoproteins.** The flavoproteins comprise a group of *yellow respiratory enzymes* isolated from animals, yeast, and higher plants. Theorell split the enzymes by dialysis into a protein fraction and a riboflavin fraction (p. 171). Neither portion alone showed enzymatic activity, but on mixing the fractions the yellow enzyme was regenerated. Later Kuhn synthesised riboflavin phosphate, one of the prosthetic groups, and on adding it to the protein fraction isolated above, enzyme activity was generated.

(d) **Nucleoproteins.** Nucleoproteins make up the greater part of the chromatin material of cell nuclei, and are therefore present in all living cells. The prosthetic group is **nucleic acid.** When either animal or plant cells are separated into nucleus and cytoplasm (*e.g.* by the flotation method of Feulgen), the nucleic acid of the nucleus is found to be different from that of the cytoplasm. Both types of nucleic acids are built up from carbohydrate, phosphoric acid, and purines and pyrimidines (p. 161), and therefore will be discussed in the next chapter. The protein portion of the nucleoproteins has not been classified. More than one protein type may be present, c.p. the nucleohistones. The protein fraction from Wheat nucleoprotein resembles leucosin, the albumin of Wheat. The plant viruses are also nucleoproteins.

(e) **Nucleotide (Pyridino-) Proteins.** A group of conjugated proteins in which the prosthetic group is related to the **nucleotides** (p. 167) contains many of the enzymes of fermentation and respiration (p. 170). Here again the *prosthetic group* controls the *type* of *reaction*—with these enzymes, reduction and phosphate transference—while the *protein portion* determines the *specificity* of the enzyme complex (that is, the *type* of *substrate*). For instance, diphosphopyridine nucleotide with the appropriate protein can effect the following oxidations: alcohol to aldehyde, aldehyde to acetate, and malate to oxalacetate.

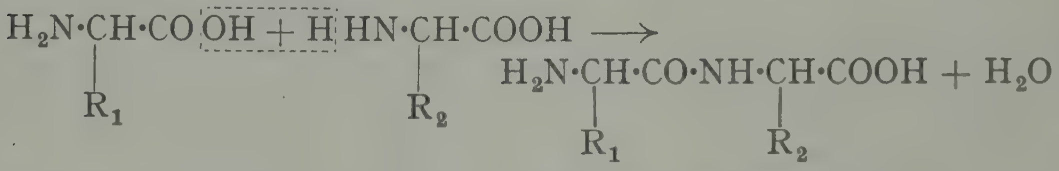
(f) **Thiamine Proteins.** Another group of enzymes can be separated into a protein portion and *diphosphothiamine.* The most important in plants is *cocarboxylase,* as it functions in respiration (p. 257). Thiamine itself is vitamin B_1 (p. 163).

(g) **Lipoproteins.** Lipoproteins are compounds in which protein and fat or lipins (especially lecithin) occur in some loose form of combination which is easily ruptured. Crude extracts of either lecithin or protein from plant tissues usually contain both. This is particularly true in the case of the *plastids,* which contain high percentages of lipin. Chibnall found that part of the protein in the *cytoplasm* of Spinach was present in a loosely combined form with substances of a fatty nature insoluble in alcohol, but it is doubtful to what extent any definite compound of lecithin and protein occurs in the plant. Similarly, Davies found that the protein of Mangolds appeared to be conjugated in the plant, and that after water extraction of the leaves of *Cruciferæ* (p. 146), further extraction with alkaline alcohol gave more protein by hydrolysing off a prosthetic group, and so rendering the protein soluble.

In animals, conjugated proteins containing carbohydrate occur, *e.g.* mucoproteins from the mucous membrane, but similar substances have not been identified in higher plants.

The Structure of the Proteins

Amino-acids are the units of which the protein molecule is composed, and they are also the ultimate products of hydrolysis of proteins. Theories as to the mechanism of the synthesis of proteins in plants will be considered in a later chapter (p. 289); for the present, the structure and properties of the proteins will be discussed. Amino-acids, as we have seen, contain both basic and acidic groups, and therefore two or more molecules can condense with the loss of water to form straight-chain compounds, called **peptides,** containing the —CONH— grouping:

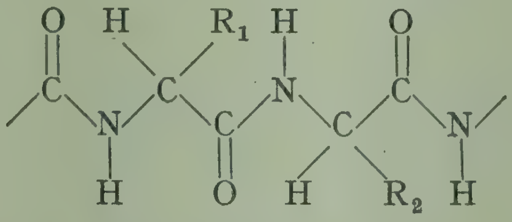


$$\mathrm{H_2N{\cdot}\underset{\displaystyle R_1}{\underset{|}{CH}}{\cdot}CO\boxed{OH + H}HN{\cdot}\underset{\displaystyle R_2}{\underset{|}{CH}}{\cdot}COOH} \longrightarrow \mathrm{H_2N{\cdot}\underset{\displaystyle R_1}{\underset{|}{CH}}{\cdot}CO{\cdot}NH{\cdot}\underset{\displaystyle R_2}{\underset{|}{CH}}{\cdot}COOH + H_2O}$$

If two molecules of glycine [$CH_2(NH_2){\cdot}COOH$] condense, the resulting compound is glycylglycine, a dipeptide, $CH_2(NH_2){\cdot}CONH{\cdot}CH_2{\cdot}COOH$. Such a compound still contains a primary amino group and a carboxyl group, and further condensation with the same or other amino-acids is possible, giving polypeptides. The evidence in favour of this **polypeptide structure** in proteins includes (*a*) the biuret reaction, due to a repetition of the —CONH— group, and (*b*) the fact that the proteins give little free nitrogen on treatment with nitrous acid. Emil Fischer synthesised many such polypeptides from amino-acids, the largest being a C_{48} compound, with a molecule derived from eighteen molecules of amino-acids. Polypeptides also are intermediate hydrolysis products of proteins, but the complexity of the protein molecule itself is much greater, as is shown by the values for the molecular weight of proteins, which is 34,500 or multiples thereof (Svedberg). Very small percentages of some of the amino-acids are obtained on hydrolysis of proteins, as there are practically infinite possibilities of combining the nineteen amino-acids when different numbers of molecules of each may take part. However, in a given protein, the constituent *amino-acids* are *arranged in definite number and sequence* in the polypeptide chain.

Bergmann introduced a new method of synthesising polypeptides, in which it was possible to use amino-acids such as lysine, which, because of a second reactive group, could not be combined by the Fischer method. Amino groups were blocked by condensation with the benzyl ester of chlorocarbonic acid (carbobenzoxy-chloride, $C_6H_5 \cdot CH_2 \cdot O \cdot COCl$); after condensation of the acid group of the amino-acid with the amino group in another (peptide formation), the carbobenzoxy group can be removed by catalytic dehydrogenation at room temperature.

X-ray examination of protein structure indicates that the molecules are in fact composed of long, but in many cases not fully extended, chains of amino-acids as in the polypeptides, with the following arrangement:—



Owing, however, to the presence of diamino and dicarboxylic amino-acids and of hydroxyl and sulphydryl groups in some of the amino-acids, cross-linkages between chains can also occur. The proteins can therefore be described as three dimensional bundles of parallel polypeptide chains, held together in folded or cyclic structures by side-chain intra-molecular bridges.

Like the amino-acids, the soluble proteins are both basic and acidic in character, owing to their retaining one or more amino and carboxyl groups uncombined in the molecule. They can therefore unite with both acidic and basic reagents to form salts, and they have an *isoelectric point.* It is significant that the isoelectric point for many of the plant proteins is fairly near the p_H value of the cell-sap. All the cytoplasmic proteins so far examined are completely precipitated at the isoelectric point, and therefore any change which would bring the cell to this point would result in its disintegration and death. However, in the region of the isoelectric point, a small change in the p_H value results in a large change in the physical properties of the proteins, such as the viscosity, and swelling, and therefore the *permeability* of the protoplasm is easily altered. With most cytoplasmic and reserve proteins of plants, the isoelectric point is at a slightly more acid value than the cell-sap, *i.e.* the protein is present in solution as the anion, and is easily extracted from such tissues. In a few plants, however, *e.g. Parthenocissus* and the Vine (*Vitis vinifera*), which have a very acid cell-sap ($p_H = 3{\cdot}0$–$3{\cdot}5$), the isoelectric point of the cytoplasmic proteins is on the alkaline side of this value, and such proteins cannot be extracted:

	Spinach	Cabbage	Rhubarb	Vine
Isoelectric point p_H . .	5·0–4·0	4·7–4·0	3·5	4·8–4·4
Cell-sap ,, p_H . .	6·57	5·60	4·00	3·02

Hydrolysis of protein with strong mineral acid (either sulphuric or hydrochloric) gives a mixture of amino-acids with some ammonia, and, if the protein be conjugated, the prosthetic group. The ammonia is formed by the hydrolysis of acid amide groups which occur in the protein molecule on the second carboxyl group of the dicarboxylic amino-acids (*vide* Amide Nitrogen, p. 138).

The first successful attempt to separate the amino-acids obtained in protein hydrolysis was made in 1901 by Emil Fischer, who fractionally distilled their ethyl esters under reduced pressure, and then hydrolysed these separate ester fractions with alkali. By this method from 65–85 per cent. of the composition of the protein molecule was determined.

Dakin showed later that the monoamino-acids can be separated as a group from the hydrolytic mixture by extraction with butyl alcohol; these are then esterified and separated by Fischer's method. The residue from the extraction yields the other amino-acids by various precipitating agents, such as phospho-tungstic acid, and through the formation of metallic salts. This method has increased the summation of the amino-acid constituents in zein of Maize to 100 per cent.

Hydrolysis of proteins by alkali is also possible, but cystine and arginine decompose, cystine losing sulphur, and arginine being split into ornithine and urea.

Bergmann used a modification of his carbobenzoxy method in the degradation of proteins. The free amino group is benzoylated, and the terminal carboxyl group ($-COOH$) is converted to the azide ($-CON_3$), which with benzyl alcohol rearranges to give a carbobenzoxy derivative ($-NH{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}C_6H_5$). Dehydrogenation removes the benzyl group, and treatment with water—which is not sufficient to hydrolyse the other peptide linkages in the molecule—splits off the terminal group of the original protein as an aldehyde.

This can be identified, and the remaining polypeptide degraded one step further. In this fashion the actual alignment of the amino-acids in the polypeptide chains can be demonstrated. A simple example of the method follows:—

Glycyl-leucyl-alanine
↓
Benzoyl-glycyl-leucyl-alanine
↓ degradation
Benzoyl-glycyl-leucine + acetaldehyde
↓ repeat degradation
Benzoyl-glycine + *iso*valeric aldehyde

The following table summarises the percentages of amino-acids obtained from some representative plant proteins:—

TABLE V.

Amino-acid.	Albumins	Globulins		Prolamins		Glutelins
	Leucosin*	Edestin	Coconut globulin	Wheat gliadin	Zein	Wheat glutenin*
Glycine	0·9	3·8	trace	0·0	0·0	0·9
Alanine	4·5	3·6	4·1	2·0	9·8	4·7
Serine	—	0·3	1·8	0·1	1·0	0·7
Valine	0·2	+	3·6	3·3	1·9	0·2
Leucine	11·3	20·9	6·0	6·6	25·0	6·0
Aspartic acid	3·6	10·2	5·1	0·8	1·8	0·9
Glutamic acid	6·7	19·2	19·1	46·1	33·8	25·7
Arginine	5·9	15·8	15·9	3·2	1·8	4·7
Lysine	2·8	2·2	5·8	0·6	0·0	1·9
Cystine	+	1·0	1·5	2·4	0·8	1·6
Phenylalanine	3·8	3·1	2·0	2·3	7·6	2·0
Tyrosine	3·3	4·5	3·2	3·1	5·9	4·3
Proline	3·2	4·1	5·5	13·2	9·0	4·2
Hydroxyproline	—	2·0	—	—	0·0	—
Histidine	2·8	2·1	2·4	2·1	1·2	1·8
Tryptophan	+	1·5	1·2	0·8	0·2	1·7
Ammonia	1·4	2·3	1·6	5·2	3·6	4·0
Total	50·4 +	96·6 +	78·8	91·8	103·4	65·3

* Estimations of some of the amino-acids from these two proteins have not yet been carried out by the newer methods of isolation.

Plant proteins differ from animal proteins not in the nature but only in the proportions of amino-acids present. The source of animal protein is eventually the amino-acids obtained from plant protein by the hydrolytic enzymes in the digestive tract; thus the amino-acid content of various plant proteins used as foodstuffs is an important criterion of their 'completeness' as nutrients. This consideration is particularly important in the case of some of the more complex amino-acids, which the animal cannot synthesise, which are nevertheless required in relatively large amounts, and in which some plant proteins are deficient. Tryptophan, cystine, and histidine, and probably tyrosine and arginine, are indispensable for the maintenance and growth of animals; lysine also is indispensable for growth, although apparently it is not required by the full-grown animal, *i.e.* for maintenance. Generally, plant proteins have a higher content of glutamic acid, arginine, and amide nitrogen than animal protein. Several plant proteins are deficient in cystine, especially the total protein (mainly globulin) of beans of the genus *Phaseolus*. Edestin (of Hemp) and Wheat gliadin and glutenin are low in lysine (*cf.* the animal protein, casein of milk, which contains 7·6 per cent. lysine), and are therefore described as *incomplete proteins*. Excelsin (of Brazil Nut) has an exceptionally high arginine content. Zein of Maize contains practically no tryptophan and no lysine, and therefore cannot support life. The **Cereal Proteins** generally contain greater amounts of glutamic acid and proline than animal protein, and are low in the basic amino-acids. The prolamin of Rice stands in sharp contrast to the others because of its high content of arginine and histidine. The **Cytoplasmic Proteins,** on the other hand, are distinguished by a high lysine content.

The estimation of 'True' protein in plant and animal tissues necessitates hydrolysis of the protein, and estimation of the various amino-acids so formed, by the methods outlined (pp. 134 and 152). As many plant tissues, however, contain only traces of nitrogen in other forms than protein, a determination of total nitrogen is usually made, especially in the analysis of foodstuffs, and a value for 'Crude' protein thus obtained. The amount of nitrogen in protein is roughly 15 per cent., and hence the percentage of crude protein is obtained by multiplying the nitrogen value by 100/15, or 6·25. The nitrogen is determined by digesting the material with concentrated sulphuric acid until all the nitrogen is converted into ammonium sulphate, and then liberating the ammonia by strong alkali, and distilling it into standard acid.

EXPT. 57. *The Estimation of Crude Protein in Pea Flour (or similar Protein Material) by Kjeldahl's Method*

About 1 grm. of the substance is weighed accurately and placed in a Kjeldahl flask (fig. 5 (A)) with 20 c.c. of concentrated sulphuric acid and a few crystals of potassium sulphate. The mixture is heated in a fume-chamber until the liquid is colourless. The cold liquid is diluted with an equal volume of water, and transferred to a round-bottomed flask, fitted with a dropping funnel and delivery tube containing a safety-bulb (fig. 5 (B)). The Kjeldahl flask is rinsed out several times with small amounts of water, and the washings added to the distillation flask. The end of the delivery tube just dips under the surface of a known volume of standard acid in a conical flask (use about 100 c.c. of *N*/10-sulphuric acid). An excess of 20 per cent. sodium hydroxide solution is introduced carefully from the dropping funnel, and the mixture heated gradually to boiling until all the ammonia has been expelled. The acid in the receiving flask is back-titrated with standard alkali (*N*/10-sodium hydroxide solution) using methyl orange as indicator, and the percentage of nitrogen corresponding to the ammonia absorbed is calculated.

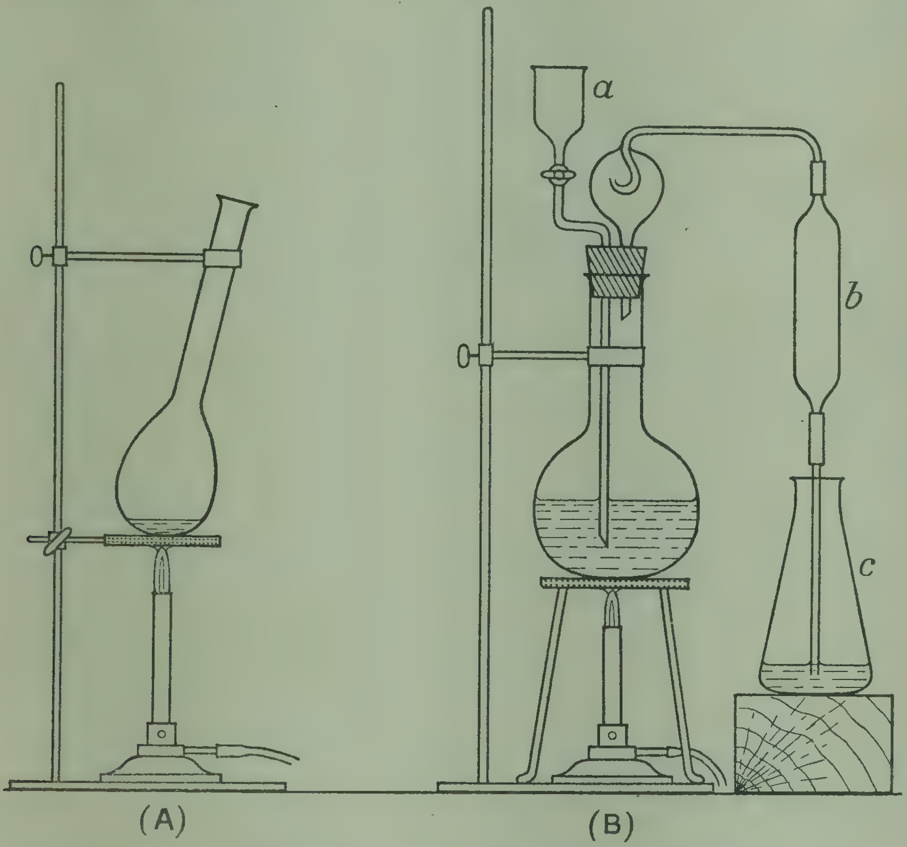


FIG. 5. Kjeldahl's Method of estimating Nitrogen

1 c.c. of *N*/10-acid corresponds to 0·0014 grm. of nitrogen.

Derived Proteins

The protein molecule can be modified by various reagents such as acids, alkalis, heat, and enzymes, giving substances intermediate in molecular complexity between the original protein and its constituent amino-acids. Such substances are classed as **Derived**

Proteins, and are subdivided into two groups, namely, the **Primary** and **Secondary Protein Derivatives.**

The **Primary Protein Derivatives** are substances which retain most of the protein characteristics in so far as they still give the colour tests of the proteins, but they are altered in *solubility* by the irreversible action of various reagents. This action is often termed the **denaturing** of proteins.

(*a*) **Proteans** are proteins which have been slightly modified by the action of acid or alkali; plant proteins readily respond to the action of acid, but alkalis seem to have less effect on plant than on animal proteins. The best example among the plant proteins is **edestan,** obtained from edestin by the action of dilute acid. Crystallised edestin dissolves in water containing a trace of hydrochloric acid, and the addition of a little sodium chloride to the resulting solution precipitates a substance which does not completely dissolve in strong salt solution (as does edestin). If the soluble part is isolated and treated with hydrochloric acid and sodium chloride as before, a further insoluble portion is obtained; this new substance, edestan, cannot be made to dissolve in neutral salt solutions. Most seed proteins give similar products, and small amounts of such proteans probably also exist naturally in seeds, owing to traces of contained acid.

(*b*) **Metaproteins** are the first hydrolytic products of the proteins —especially of the albumins and globulins—produced by the action of acid or alkali. They are insoluble in neutral solvents but soluble in dilute acids and alkalis. The first action of nitric acid in both the Xanthoproteic test and Millon's reaction is often to produce a white precipitate of metaprotein (*vide* experiments, pp. 144 and 145).

(*c*) **Coagulated Proteins** are produced by the action of alcohol, heat, or heavy metals and their salts on the simple proteins. Zein, which is soluble in aqueous alcohol, is precipitated as a gel by absolute alcohol. Leucosin, the albumin from Wheat, is coagulated when heated in aqueous solution (p. 144; *cf.* egg-white in animal proteins), and many of the seed proteins are precipitated irreversibly by metallic salts, *e.g.* copper acetate.

The **Secondary Protein Derivatives** are all compounds formed at various stages in the hydrolysis of the proteins by acids, alkalis, or enzymes, in the following sequence:—

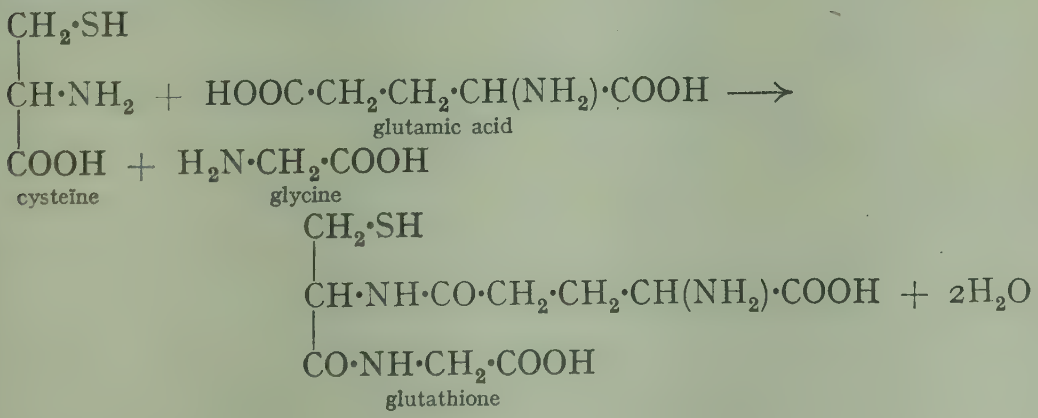
$$\text{Proteins} \longrightarrow \text{Metaproteins} \longrightarrow \text{Proteoses} \longrightarrow$$
$$\text{Peptones} \longrightarrow \text{Peptides} \longrightarrow \text{Amino-acids.}$$

(*a*) **Proteoses** are soluble in water, not coagulated by heat, but, like the proteins, precipitated from solution by saturation with ammonium sulphate. Proteoses occur in different parts of various plants, and are obtained in many plant juices and extracts, especially from seeds of Lupin, Vetch, Hemp and Flax, and in Wheat embryo (p. 143). They are in some cases actual components of the plant tissue concerned, but sometimes they may be formed by the action of proteolytic enzymes during extraction.

(*b*) **Peptones,** like the proteoses, are soluble in water, and give most of the colour reactions of the proteins, but they are not precipitated from solution by ammonium sulphate. They, too, occur in small amounts in plant tissues, and are formed especially during the germination of seeds rich in protein, *e.g.* Lupin seeds. They are probably not translocation products, as they are hydrolysed further to amino-acids for this purpose, although they may diffuse through a few neighbouring cells.

(*c*) **Peptides** or **Polypeptides** are straight-chain compounds of the amino-acids (p. 150), condensed by the loss of water and the formation of the peptide linkage —CONH—. They occur naturally in small amount in seeds, and are obtained in the hydrolysis of the proteins. Some of them are very resistant to further degradation to the component amino-acids; *e.g.* a dipeptide of proline and phenylalanine, and the dipeptide *l*-leucyl-*d*-glutamic acid have been isolated from the products of hydrolysis of Wheat gliadin under different conditions, and the latter compound is identical with one of Fischer's synthetic dipeptides. The properties of the peptides depend on their complexity, and on the amino-acids present in the molecule. The peptides are all soluble in water, and some of them give the colour reactions of the proteins, especially the biuret test.

Glutathione, discovered by Hopkins in 1921, is one of the most important peptides, as in the animal world it is intimately connected with the respiration process. It has also been obtained in crystalline form from germinating Peas. Its possible relation to plant respiration will be dealt with later (p. 286). Glutathione is the tripeptide γ-glutamyl-cysteïnyl-glycine:



$$\begin{array}{l}
CH_2\cdot SH \\
| \\
CH\cdot NH_2 + \underset{\text{glutamic acid}}{HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH} \longrightarrow \\
| \\
\underset{\text{cysteïne}}{COOH} + \underset{\text{glycine}}{H_2N\cdot CH_2\cdot COOH} \\
\qquad\qquad CH_2\cdot SH \\
\qquad\qquad | \\
\qquad\qquad CH\cdot NH\cdot CO\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH + 2H_2O \\
\qquad\qquad | \\
\qquad\qquad \underset{\text{glutathione}}{CO\cdot NH\cdot CH_2\cdot COOH}
\end{array}$$

Just as the amino-acid, cystine, is built up from two molecules of cysteïne by the loss of two atoms of hydrogen, so glutathione, by reason of its sulphydryl group (–SH), can be oxidised so as to yield a condensation of two molecules. Using the symbol G for the rest of the molecule attached to the sulphydryl group, the oxidation proceeds according to the following equation:—

$$2\text{G—SH} + \text{O} \longrightarrow \text{G—S—S—G} + \text{H}_2\text{O}$$

Pantothenic Acid, the vitamin of the B complex which prevents dermatitis in chickens (Williams), contains a polypeptide linkage between β-alanine and α-, γ-dihydroxy-β, β′-dimethyl-butyric acid. It has been isolated from yeast, Rice bran, and Sugar-cane, and has the formula: $CH_2OH{\cdot}C(CH_3)_2{\cdot}CHOH{\cdot}\mathbf{CONH}{\cdot}CH_2{\cdot}CH_2{\cdot}COOH$.

Proteolytic Enzymes

The hydrolysis of protein in stages can be effected by enzymes, which are grouped together as **proteases** or **proteinases.** They are widely distributed in plants. One of the major activities of the germinating seed is the enzymatic hydrolysis of the reserve protein, with the ultimate formation of soluble products, including the amino-acids, which are then translocated to the growing points for the synthesis of new protoplasm. Plant proteolytic enzymes were originally compared with those of animal origin, which are concerned with the digestion of protein, *viz.* pepsin, trypsin, and erepsin, but none of the plant enzymes correspond to this classification. Then it was found that some of the enzymes acted on proteins and others on peptides, so they were classified as proteinases and peptidases. However, after Bergmann determined the arrangement of amino-acids in some proteins, and synthesised peptides of amino-acids arranged in a known order, it became clear that the enzymes all hydrolyse the peptide linkage (—CONH—) in both peptides and proteins of types specific for each enzyme. The selective action of the enzymes is determined not by the difference in the length of the amino-acid chain between simple polypeptides and high molecular weight proteins, but by the nature of the *side-chains* or reactive groups on the polypeptides, and by their position relative to the peptide linkage being hydrolysed. The enzymes are therefore now divided into two groups: (*a*) **exopeptidases,** which act only on *terminal peptide linkages*, and (*b*) **endopeptidases,** which hydrolyse *peptide bonds* located *within* the long peptide chain. Thus the same polypeptide of known structure can be hydrolysed at different peptide linkages by different enzymes.

For the (*a*) group, the substrate must also have a free α-amino or α-carboxyl group adjacent to the terminal peptide linkage to be hydrolysed. The enzymes are therefore sometimes subdivided into aminopeptidases and carboxypeptidases, but many exopeptidases are not specific for one particular side-chain. Exopeptidases occur in plant tissues; they are present in leaves, fruits, roots, bulbs, and both resting and germinating seeds. Their hydrolytic action can be detected either by the increase in amino-nitrogen content, or by the tryptophan-bromine reaction on the addition of an aqueous extract or simply of the crushed tissue to an artificial supply of peptone. Exopeptidases have been isolated from Hemp seed (*Cannabis sativa*) and from Cabbage leaves (*Brassica oleracea*).

(*b*) The endopeptidases are difficult to classify, and so the original names of the enzymes are retained, *e.g.* papain, bromelin, etc. Many of these are derived from the *latex* of the plants, *e.g.* **papain** from the latex of the green fruit of *Carica Papaya*, and **ficin** (cradein) from the Fig (*Ficus*), but others are intracellular tissue enzymes, *e.g.* **bromelin** from Pineapple (*Ananas sativus*). These three enzymes and several others from tropical plants are inactivated by mild oxidation, and reactivated by sulphydryl compounds and hydrocyanic acid. As there are traces of sulphydryl compounds in all plant tissues, they may function as natural coenzymes (p. 254). Papain, bromelin, and **asclepain** (from the latex and roots of several Milkweeds (*Asclepias*)) have been crystallised, and they themselves are **proteins.** They appear to act on peptide bonds at a specific amino-acid grouping. This has been shown especially for the corresponding animal enzymes, pepsin and trypsin, the former requiring the presence of *L*-tyrosine and the latter *L*-lysine in the substrate. Other endopeptidases are widely distributed in the vegetative parts of plants and in germinating seeds, and have been investigated particularly in the Cereals and the *Leguminosæ.* Their action may be demonstrated by autolysis of the plant tissue, and either measuring the amount of *amino* nitrogen, which is found to increase, or by testing for the production of tryptophan, the most easily detected amino-acid.

A special group of proteinases resemble animal pepsin, as they too hydrolyse *animal* protein. They occur in insectivorous plants, such as the Sundew (*Drosera*), the Pitcher plant (*Nepenthes*), and the American plant, Venus' Fly-trap (*Dionæa*): these all utilise the protein of the trapped insects as a source of *nitrogen.* The method of trapping the insects varies in the different plants, but in all cases there follows digestion of the protein by the secretion of an acid fluid containing the pepsin-like enzyme, and the peptones so formed are absorbed by the tissue of the leaf.

A further type of enzymatic decomposition of proteins and their derivatives is possible, namely, the splitting of the C—N linkage other than hydrolysis of the —CONH— linkage. This may involve (*a*) **deamination,** the removal of an amino-group as ammonia (p. 135), and these deaminases, or more correctly **transaminases** (for they can also catalyse the reverse reaction) are widely distributed in plants; and (*b*) **deamidation,** or hydrolysis of an acid amide group. This is exemplified by the cleavage of the special compound **arginine** into ornithine and urea by **arginase,** found in the expressed juice and seedlings of Lupin and in seedlings of Vetch (p. 136). *Asparaginase,* found in germinating Barley, is sometimes placed in this group. It splits off ammonia from asparagine and glutamine with the formation of aspartic and glutamic acids respectively. But this reaction appears to be a special case, as the enzyme also hydrolyses simple peptides such as glycylglycine. It is therefore more correctly an exopeptidase.

CHAPTER XVI

CYCLIC UREÏDES AND PURINES. NUCLEIC ACIDS

THERE exists a group of **heterocyclic** compounds containing carbon, hydrogen, nitrogen, and usually oxygen, whose heterocyclic structure, though stable, differs from that of substances like the alkaloids and the anthocyanin pigments in that the latter types show aromatic properties, whereas the former do not. Among such compounds are the **cyclic ureïdes** and **purines,** some of which are found free in plants, and, of more importance, are universally distributed in living matter as part of the more complex molecule of the **nucleic acids.**

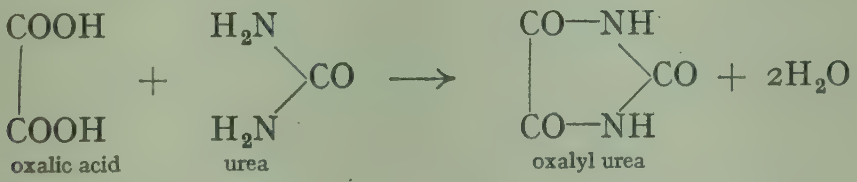
CYCLIC UREÏDES

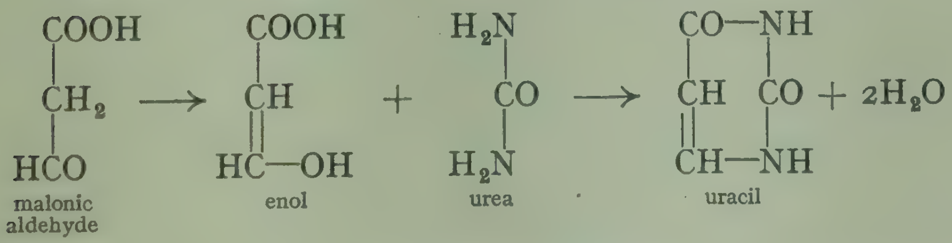
Structure. In the same way as acid amides may be considered as structurally derived from acids by the condensation of the hydroxyl group with ammonia (*a*), so the ureïdes are derived from acids by condensation with *urea* (*b*):

$$(a)\quad R{\cdot}COOH + NH_3 \longrightarrow \underset{\text{amide}}{R{\cdot}CO{\cdot}NH_2} + H_2O$$

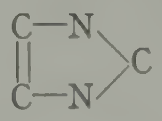
$$(b)\quad R{\cdot}COOH + \underset{\text{urea}}{H_2N{\cdot}CO{\cdot}NH_2} \longrightarrow \underset{\text{ureïde}}{R{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}NH_2} + H_2O$$

If the ureïdes of *dibasic acids* are formed, cyclic compounds result, *e.g.* oxalic acid gives rise to a five-membered ring, whereas malonic acid or malonic aldehyde acting through the enol form give six-membered rings:

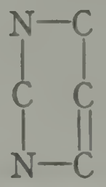




These two compounds are representatives of two cyclic structures based on the **iminazole** or glyoxaline nucleus, and the **pyrimidine** nucleus:



Iminazole nucleus



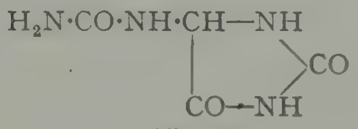
Pyrimidine nucleus

Properties. Compounds containing the grouping

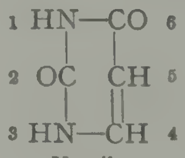
—CO—NH—CO—

possess *acidic* properties, the hydrogen atom attached to the nitrogen being replaceable by metals, forming metallic salts. The nitrogen atom itself retains some of its basic properties, and may still form salts with acids, *e.g.* with picric acid, and may undergo condensation with other molecules, *e.g.* with the active hydroxyl group in sugars, forming a type of glycoside called a **nucleoside.** This distinctive grouping can exist in both keto and enol forms, called in the case of the —NH—CO— grouping the *lactam*, —CO·NH—, and *lactim*, —C(OH):N—, forms.

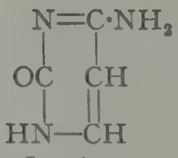
Iminazole Derivatives. The iminazole ring has been met with already in the amino-acid **histidine** (p. 131). A substance called **allantoïn,** a diureïde from two molecules of urea and one of glyoxalic acid, occurs in a number of plants, including Beets, Peas, Beans, the rhizome of the Comfrey (*Symphytum officinale*), and shoots of the Plane Tree (*Platanus orientalis*).



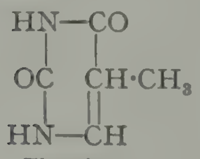
Allantoïn



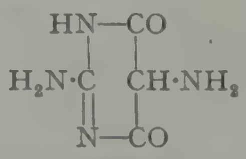
Uracil



Cytosine



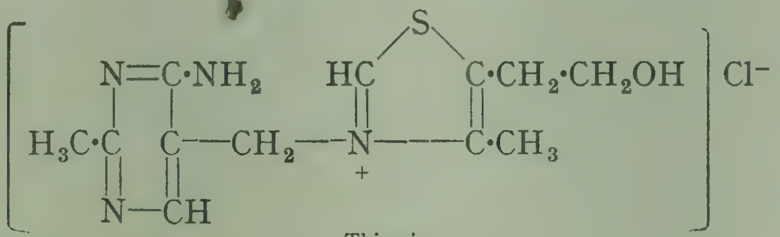
Thymine



Divicine

Pyrimidine Derivatives. Three pyrimidine derivatives are obtained by the decomposition of nucleic acids, *viz.* **uracil, cytosine,** and **thymine;** uracil and cytosine are obtained from the plant nucleic acids, cytosine and thymine from the animal ones. A synthesis of uracil from malonic aldehyde has been shown above, the malonic aldehyde being prepared in the enol form in the reaction mixture by acting on malic acid with concentrated mineral acid. Malic acid is common in plants, and is also the hydroxy-acid corresponding to aspartic acid, hence it has been suggested that a similar mechanism accounts for the synthesis of uracil in the plant. Uracil, $C_4H_4O_2N_2$, according to the given system of numbering the pyrimidine nucleus, is 2, 6-dioxypyrimidine. Cytosine, $C_4H_5ON_3$, is 2-oxy-6-amino-pyrimidine, or the amino-derivative of the lactim form of uracil, while thymine, $C_5H_6O_2N_2$, is 5-methyl-uracil or 2, 6-dioxy-5-methyl-pyrimidine. All three are soluble in hot water, and all act as **acids,** forming silver salts which are soluble in acid solution. Uracil and thymine also dissolve in alkali with the formation of alkali salts, whereas cytosine is more basic, and is distinguished from the other two by forming an insoluble salt with phosphotungstic acid. **Divicine,** $C_4H_6O_2N_4$, which is 2, 5-diamino-4, 6-dioxy-pyrimidine, occurs combined with *glucose* as the nucleoside **vicine.** Vicine is not a component of any plant nucleic acid which has been isolated, but it occurs in seeds, particularly those of species of *Vicia;* its principal source is Vetch meal.

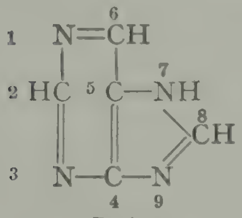
Thiamine. When a sulphur atom replaces one of the nitrogen atoms in the iminazole nucleus, the **thiazole** nucleus is formed. In both plants and animals the most important compound containing this nucleus is thiamine, which contains in addition a pyrimidine ring. Isolated as the hydrochloride, thiamine is the anti-beri-beri and anti-polyneuritis **vitamin B_1** (aneurin). Its structure has been proved by synthesis. It is present in greatest amount in nuts and seeds; in the Cereal grains its highest concentration is in the portion lost in the milling process, and hence synthetic thiamine is added to milled flours. The leaves of all plants examined were found to contain thiamine in very small amount, but in approximately constant concentration. Diphosphothiamine or thiamine hydrochloride pyrophosphate (which has also been synthesised) is **cocarboxylase,** the coenzyme which, conjugated with protein, forms carboxylase. This is the enzyme of yeast and higher plants which liberates carbon dioxide in the fermentation and respiration processes (pp. 257 and 281). In the coenzyme, the two phosphate groups form a pyrophosphate ester with the primary alcoholic group of thiamine. Thiamine also occurs in yeast as thiamine disulphide pyrophosphate.



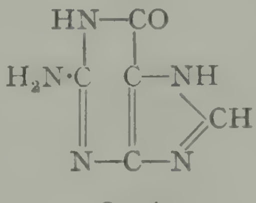
Thiamine

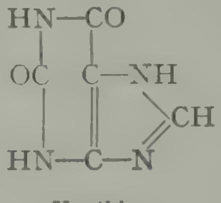
PURINES

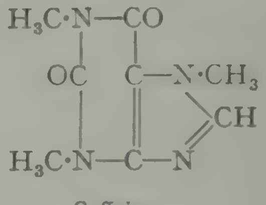
The purines also are cyclic ureïdes, the purine nucleus consisting structurally of a condensed pyrimidine and iminazole ring; the theoretical parent substance is purine:



Purine


Guanine


Xanthine


Caffeine

Both plant and animal nucleic acids contain two purines in the molecule, **adenine** and **guanine.** These also occur in plant and animal tissues, both free and as nucleosides; so also do **hypoxanthine** and **xanthine.** Three purines are peculiar to plants, **caffeine, theobromine,** and **theophylline,** while **uric acid** is characteristic of animals. Purines, like the pyrimidines, show amphoteric properties, the hydrogen of the —NH group being acidic, and if they contain the —NH·CO— grouping they can exist in lactam and lactim forms.

Adenine, $C_5H_5N_5$, is 6-amino-purine, while **guanine,** $C_5H_5ON_5$, is 2-amino-6-oxy-purine. Adenine and guanine are both amphoteric; they are soluble in alkalis, ammonia, and mineral acids. Adenine is also soluble in acetic acid, while guanine is not; salts of guanine with acids are hydrolysed in water. Adenine and guanine are precipitated as their hydrochlorides by hydrolysis of nucleic acid in methyl alcohol with hydrogen chloride. Adenine has also been isolated from Beet, Sugar-cane, and seedlings of the *Leguminosæ.* **Vernine,** a nucleoside of guanine with the pentose *d-ribose,* occurs in Beet, in seedlings of *Vicia,* and in seeds of Lupin and of *Trifolium pratense,* while a nucleoside of adenine with a sugar containing sulphur, which appears to be a thiomethylpentose, has been isolated from yeast.

Hypoxanthine, $C_5H_4ON_4$, or 6-oxy-purine, and **xanthine,** $C_5H_4O_2N_4$, or 2, 6-dioxy-purine, are probably formed in nature from adenine and guanine, respectively, by the replacement of an amino-group by hydroxyl. Enzymes termed *adenase* and *guanase* have been isolated, *e.g.* from Lupin seedlings, which catalyse these transformations. Hypoxanthine is widely distributed in plants, and has been detected in Beet, Potato, seedlings of Lupin, and seeds of Barley, Mustard, Pepper, and Melon. A nucleoside of hypoxanthine with ribose, called **inosine,** has been found in Beet and in yeast. Xanthine occurs in Beet, leaves of Tea, and seedlings of Vetch and Lupin. **Uric acid,** which is 2, 6, 8-trioxy-purine, is the ultimate oxidation product of these purines in the metabolism of reptiles, birds, and some mammals. Uric acid, and some other purines, *e.g.* xanthine and caffeine, are readily detected by the *murexide test.* If the purine is evaporated to dryness with concentrated nitric acid, a yellow residue is obtained which turns purple on addition of ammonia.

Caffeine, theobromine, and theophylline are all xanthine derivatives occurring only in plants; they have a definite physiological action, including that of stimulating the central nervous system. They occur in beverages, *e.g.* tea, coffee, cocoa. They all show amphoteric properties, are neutral to litmus, and have a bitter taste. **Theobromine,** $C_7H_8O_2N_4$, or 3, 7-dimethyl-xanthine, is found in Cacao beans (*Theobroma Cacao*) to the extent of about 2 per cent. It also occurs in smaller amounts in leaves of the Tea plant (*Thea sinensis*) and in West African Kola nuts (*Cola acuminata*). **Theophylline,** $C_7H_8O_2N_4$, is an isomer of theobromine, being 1, 3-dimethyl-xanthine. It occurs in Tea leaves. **Caffeine,** $C_8H_{10}O_2N_4$, is 1, 3, 7-trimethyl-xanthine. It occurs in Tea leaves (1–5 per cent.), Coffee beans (1–1·5 per cent.), Kola nuts (2·7–3·6 per cent.), in the South American 'mate' or 'Paraguay tea' from the leaves of *Ilex paraguensis* (1·2–2 per cent.) and in 'guarana' from the fruit of *Paullinia cupana* (3–5 per cent.).

Nucleic Acids

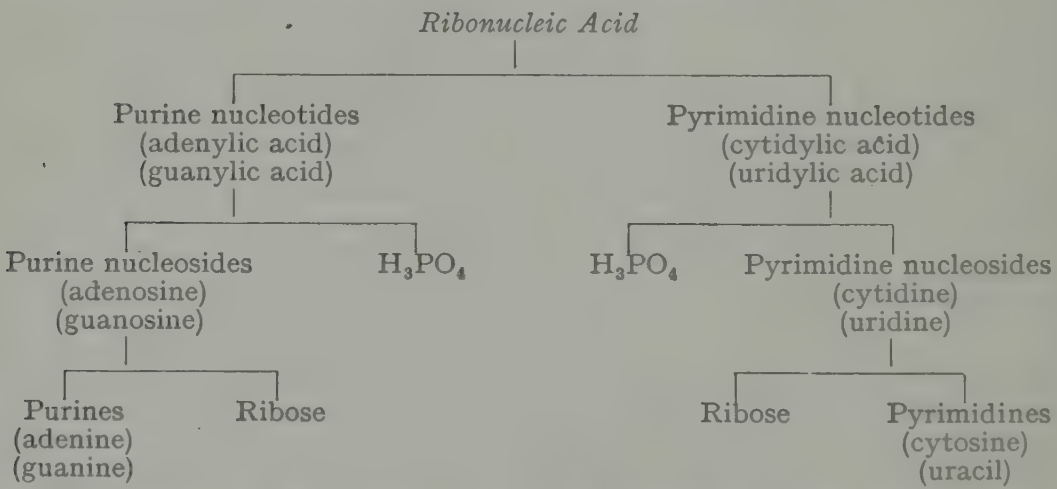
Early attempts to master the secret of living matter led to investigations of the material which comprised the *chromatin* of **cell nuclei.** This material is now regarded as **nucleoprotein** (p. 149). It occurs in both the nucleus and the cytoplasm; in fact, chromosomes appear to be nucleoproteins. So are the plant viruses. Early attempts to isolate nucleoproteins resulted in the removal of part of the protein by hydrolysis, and the separation of a substance of indefinite composition which still contained protein and which was called 'nuclein' by the early investigators (Meischer, 1869). It was later shown that substances free from protein could be prepared from these nucleins, and that they were insoluble in water and dilute acids, had strong acidic properties, were soluble in alkalis, and could be isolated as metallic salts. They contained some nitrogen and a relatively high percentage of phosphorus. These substances were termed **nucleic acids.** Nucleic acids from animal tissues (especially from the thymus gland), from yeast, and from grain embryos (Wheat, Rye) have all been studied. By the work of Kossel, Jones, Levene, and Gulland, the structure of the nucleic acids has been gradually elucidated.

Structure. Two distinct types of nucleic acids, differing in the sugar component of the molecule, have been isolated (Faulken) from normal cells. One form, **desoxyribonucleic acid,** is present in all *cell nuclei*, while the other, **ribonucleic acid,** has been isolated from the *cytoplasm* of both plant and animal cells, although smaller quantities also exist in the nucleolus. The separation of these two types has been accomplished in animal cells, in yeast cells, and in cells of higher plants, *e.g.* Rye embryos (Behrens). The original division of nucleic acids into plant and animal forms is therefore no longer valid. These two acids on complete hydrolysis yield the following products:—

Desoxyribonucleic acid (Nuclei)		Ribonucleic acid (Cytoplasm)
Phosphoric acid		Phosphoric acid
D-2-Desoxyribose	*sugar*	*D*-Ribose
Guanine Adenine	*purines*	Guanine Adenine
Cytosine Thymine	*pyrimidines*	Cytosine Uracil

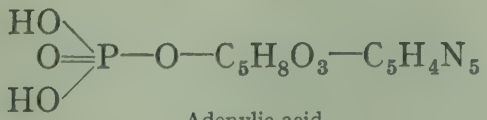
It will be seen that the two groups also differ in one of the pyrimidine components, cytoplasm nucleic acid (from yeast) containing uracil, whereas nuclear nucleic acid (from the thymus gland) contains thymine. *Tobacco mosaic virus*, on the other hand, appears to contain an optical isomer of the uracil isolated from yeast.

Selective hydrolysis of nucleic acids gives a variety of products which help to elucidate the mode of linking of these varied groups in the molecule. This is represented schematically below:



Nucleosides. The nucleosides are *glycosides* of the purines and the pyrimidines (p. 162). When ribonucleic acid is heated under pressure with concentrated ammonium hydroxide, four nucleosides, **adenosine, guanosine, cytidine,** and **uridine,** are obtained, together with four molecular proportions of phosphoric acid. These nucleosides can be further hydrolysed by *acids* to the sugar, ribose, and the respective purines and pyrimidines. The pyrimidine nucleosides are much more difficult to hydrolyse, and this gives a method of separating the two groups. A phosphatase enzyme, *ribonuclease,* from sweet Almonds, also brings about the hydrolysis of ribonucleic acid; it carries the hydrolysis to the first stage. Other enzymes termed *nucleosidases* have been isolated from various tissues which hydrolyse the nucleosides to sugar and the aglucones; some hydrolyse the purine nucleosides preferentially, others the pyrimidine nucleosides. That the ribose molecule is attached to either position 7 or 9 (see formula, p. 164; the tautomeric modification with the >NH group in the 9 position is also possible), through elimination of water with the hydrogen on the nitrogen atom, is shown by the fact that adenosine can be converted into the corresponding nucleoside of hypoxanthine, and guanosine into xanthosine. Comparison of the ultra-violet adsorption spectra of the natural compounds with synthetic 7- and 9-derivatives shows that the union is at the 9-position. Similarly in the pyrimidine nucleosides, the point of union is position 3 (p. 162). The nucleosides are all weak acids, dissolving to some extent in water, and readily in strong alkalis. They form salts with lead and silver. Some of them are amphoteric, and form salts with acids, especially picric acid. Other nucleosides besides those derived from the nucleic acids have been found in the free state in plants, *e.g.* vernine and vicine, and Emil Fischer prepared several synthetic nucleosides.

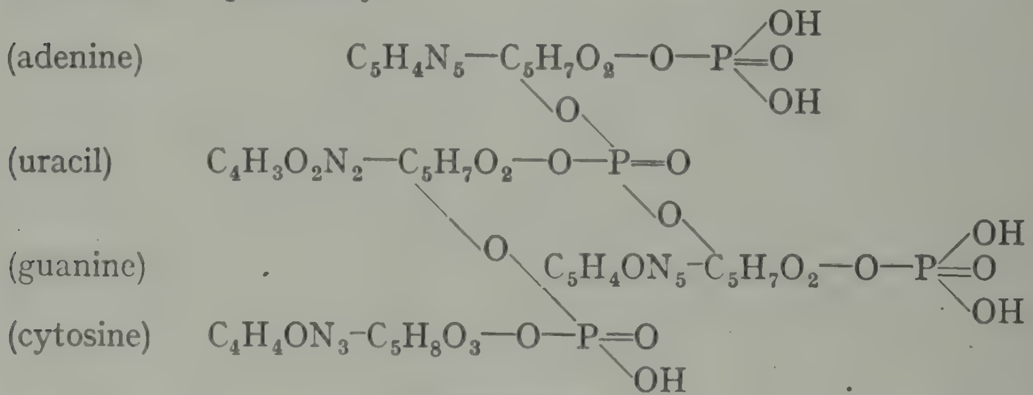
Nucleotides. Still more selective hydrolysis of ribonucleic acid, *viz.* by the action of ammonia at 105–115° C. or by 1 per cent. sodium hydroxide at room temperature, results in four different nucleotides, **adenylic, guanylic, cytidylic,** and **uridylic acids.** The nucleotides are *phosphoric esters of the nucleosides*, the phosphoric acid being combined in an ester linkage with the sugar residue in the symmetrical 3-position. This is shown by the fact that adenylic acid, on hydrolysis with alkali—which readily hydrolyses ester linkages—yields phosphoric acid and the nucleoside adenosine; while on hydrolysis with dilute acid the glycosidic linkage is ruptured, and adenine and the phosphoric ester of ribose are the products:



$$\begin{matrix}\mathrm{HO}\diagdown & \\ \mathrm{O}{=}\mathrm{P}-\mathrm{O}-\mathrm{C_5H_8O_3}-\mathrm{C_5H_4N_5} & \\ \mathrm{HO}\diagup & \end{matrix}$$

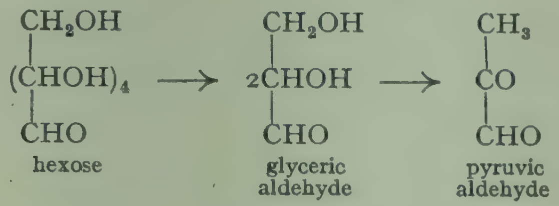
Adenylic acid

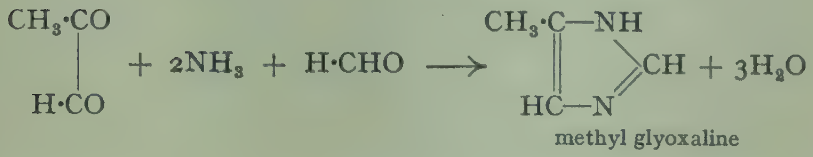
Nucleic Acid. Nucleic acids are **polynucleotides,** probably of varying molecular complexity. The existence of a **tetranucleotide** has been definitely established, but others of higher orders also exist. The molecular weight of a tetranucleotide is 1400, whereas the molecular weight of yeast and thymus nucleic acids is 2×10^4; that is, they contain about 15 tetranucleotide units. Nucleic acid from Tobacco mosaic virus is even more complex, as its molecular weight is of the order of 3×10^{15}. Hydrolysis of nucleic acid to mononucleotides gives an increase in acidity, and hence the strongly acidic hydroxyl groups of some of the phosphoric acid residues must be involved in the linkage of the mononucleotides to give the polynucleotides. There are, in fact, three types of linkage possible between mononucleotides by the elimination of water: (i) between phosphoric acid hydroxyl groups, (ii) between carbohydrate hydroxyls, *i.e.* an ether linkage, (iii) between a hydroxyl of the phosphoric acid of one nucleotide with the hydroxyl on the sugar of an adjacent nucleotide, *i.e.* an ester linkage. Levene found that titration constants indicated a phosphoric acid—sugar linkage (type iii), the phosphoric acid being cross-linked to carbon atom number 2 in the sugar of the adjacent nucleotide. For each four atoms of phosphorus, there appears to be one phosphoryl group which is triply linked; the following formula for a tetranucleotide is therefore a possibility:


(adenine)
$C_5H_4N_5—C_5H_7O_2—O—P$
OH
O
OH
O
(uracil)
$C_4H_3O_2N_2—C_5H_7O_2—O—P=O$
O
O
(guanine)
$C_5H_4ON_5—C_5H_7O_2—O—P$
OH
O
OH
(cytosine)
$C_4H_4ON_3—C_5H_8O_3—O—P=O$
OH

Ribonucleic acid

Synthesis of Nucleic Acid in the Plant. The nucleic acids are intimately connected with cell division; their synthesis must, indeed, be one of the first chemical changes taking place in the plant. The acids are structurally derivable from *sugar*, *ammonia*, and *phosphoric acid*, and these substances must be the original products responsible for their synthesis, the stages of which are not known. It can be shown in the laboratory, however, that under special conditions ammonia will act on a variety of sugars, including glucose and fructose, to give methyl-glyoxaline, which contains the iminazole ring The reaction is explained by the splitting of the sugar molecule to glyceraldehyde, which is then reduced to pyruvic aldehyde (methyl glyoxal), and this is acted on by ammonia and formaldehyde as follows:—





Glyceraldehyde is an intermediate product in respiration (p. 256) and formaldehyde is formed in plants by various decomposition reactions.

Another conceivable method of synthesis of the iminazole, pyrimidine, and purine rings in the plant is by the action of urea on simple carbon compounds, two molecules of urea and one of pyruvic aldehyde, for instance, giving a purine ring. Examples of this kind have already been given (p. 161), and the possibility of urea occurring in the plant as a metabolic product is envisaged by the wide distribution of urease (p. 139) and by its ability to synthesise urea from ammonium carbonate. *Artificial nucleoproteins* have been formed by precipitating a mixture of protein and nucleic acid with acetic acid. It was found that the protein could bind varying amounts of nucleic acid up to 25 per cent. of its own weight, this amount being dependent on the number of free amino groups in the protein.

Physiological Function. Nucleic acids are colloidal substances of high molecular weight, and are amphoteric, with the acidic properties predominating. In common with acidic colloids of high molecular weight, nucleic acids, according to Hammarsten, are able to regulate osmotic pressures by attracting basic ions, the molecular volume of which is so small that they are included within the molecular volume of the colloid, and hence the osmotic pressure is lower than the ionic concentration of the solution demands. There is little doubt that, being easily affected by electrolytes, the nucleic acids perform a regulating function in the nucleus. Some (probably all) naturally-occurring nucleoproteins migrate as a single molecular entity to the same electrode, indicating that they are not merely salts of protein and nucleic acid, but what the linkage actually is has not yet been shown. Cytoplasmic ribonucleoproteins constitute a prime source of synthesis, since they contain enzymes both of the proteinase type (p. 158) and of the oxidation-reduction series (adenylic acid phosphates). The fact that the plant viruses and the genes, which are both nucleoproteins, are capable of self-duplication suggests the fundamental role played by the nucleic acids in all living processes.

COENZYMES

Of the conjugated proteins (p. 148) which function as enzymes, two types of prosthetic groups are related to the nucleic acids, *viz.* **adenylic acid coenzymes** and the **flavins** of the flavoproteins.

Adenylic Acid Coenzymes

The adenylic acid coenzymes fall into two groups: (*a*) the **adenine nucleotides,** which are coenzymes of *phosphate transfer*, and (*b*) the **phosphopyridine nucleotides,** which are coenzymes of *hydrogen transfer*. In group (*a*), an adenylic acid (p. 167) in which the phosphoric acid is combined with adenosine in the terminal position of the ribose portion of the molecule, *i.e.* adenosine-5′-phosphoric acid, combines with one or two additional phosphoric acid groups to give **adenosine diphosphate** (**ADP**) and **adenosine triphosphate** (**ATP**) (p. 255). The dinucleotide, **di-(adenosine-5′-phosphoric acid),** in which the phosphoric acid of one nucleotide molecule is cross-linked to the ribose in the other nucleotide molecule, has been isolated from yeast and from seeds. It is a *cophosphorylase*, accepting two molecules of phosphoric acid from two molecules of phospho-pyruvic acid to yield pyruvic acid and a di-(adenosine-tetraphosphoric acid).

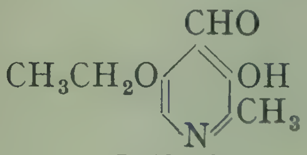
In group (*b*), adenosine-5′-phosphoric acid is united through a second phosphoric acid group with *nicotinamide.* Here the tertiary nitrogen of the pyridine ring (p. 128) is engaged. The coenzymes are therefore often described as phosphopyridine nucleotides, and cozymase I (from yeast) is a **diphosphopyridine nucleotide** (**DPN).** The arrangement of the groups may be indicated as follows:

adenine-ribose-phosphate-phosphate-ribose-nicotinamide

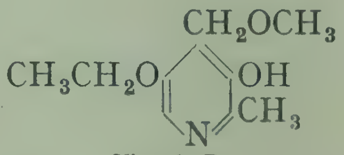
A triphosphate of this type occurs in enzymes from animal sources. They are coenzymes of hydrogen transfer (p. 260), the nicotinamide portion of the molecule being capable of reversible oxidation and reduction.

(*c*) Related to nicotinamide, but not to the nucleotides, are the prosthetic groups of another series of enzymes. **Pyridoxal** is 2-

methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine, and **pyridoxamine** is the corresponding 4-aminomethyl-compound. Several coenzymes are phosphoric esters of these pyridine derivatives, *e.g.* glutamic acid codecarboxylase is pyridoxal phosphate. *Transaminase* from plants (p. 290) uses these pyridine derivatives to transfer the amino group between amino-acids and α-keto-acids. The relationship between vitamins and enzymes is apparent, as **vitamin B_6** (aneurin) is pyridoxine (2-methyl-3-hydroxy-4, 5-dihydroxymethyl-pyridine):



Pyridoxal

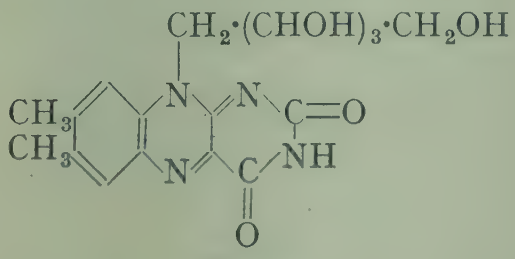


Vitamin B_6

These three groups are sometimes referred to as the 'pyridinoproteins', but this name only connotes groups (*b*) and (*c*); the term 'nucleotide proteins', on the other hand, properly belongs only to groups (*a*) and (*b*).

Flavin Coenzymes

The *yellow enzymes* are conjugated proteins in which the prosthetic group is always derived from **riboflavin.** In structure this is comparable to a nucleotide, being a compound of D-*ribose* with a complex nitrogenous base containing an iso-*alloxazine* nucleus. When the prosthetic groups of the yellow enzymes were identified, riboflavin was already known as **vitamin B_2**, a dietary factor essential to the nervous system. It has been synthesised. It is probably universally distributed in plant and animal cells. In plants, it is present in highest concentration in the leaves, and from a nutritional standpoint green vegetables such as Spinach, Lettuce, and Broccoli provide the greatest amount of riboflavin available from plant sources. Riboflavin is 6, 7-dimethyl-9(*d*-1-ribityl)-*iso*alloxazine:



Riboflavin

Riboflavin can add reversibly two hydrogen atoms to the two tertiary nitrogen atoms, with a shift in the double bonds; the reduced compound is colourless.

In the coenzymes, riboflavin is always combined with phosphoric acid. Warburg's 'yellow respiratory enzyme' from yeast contains riboflavin-5′-phosphoric acid. Other flavin coenzymes are comparable to the dinucleotides, being iso-**alloxazine-adenine-dinucleotides.** Most of these enzymes are of animal origin, but some of them have also been detected in plants, *e.g.* d-*amino oxidase* and *xanthine oxidase.* The coenzyme is built up as follows:

riboflavin-phosphate-phosphate-ribose-adenine

Hence it is closely related to cozymase, in which the flavin is replaced by nicotinamide. By virtue of the *iso*-alloxazine portion of the molecule, all the flavin enzymes can add on hydrogen to form a leuco compound, thereby oxidising a substrate. The yellow enzyme is then regenerated from the leuco form by oxidation, usually through the medium of another enzyme system.

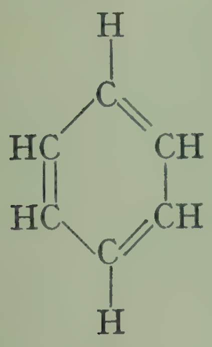
PART VI. CYCLIC COMPOUNDS

CHAPTER XVII

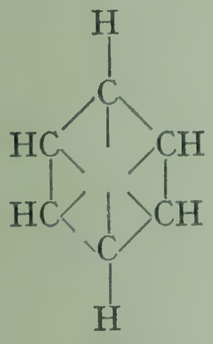
AROMATIC COMPOUNDS. PHENOLS. INOSITOLS

Although the term 'aromatic' is now reserved for compounds containing a cyclic structure of carbon atoms with benzene as the parent substance, it was first used for the fragrant oils and resins found in various parts of plants, especially flowers, buds, and leaves, and in the exudations from the trunks of trees after injury. Many of these compounds, such as the chief constituents of oils of cloves and cinnamon, are in fact benzene derivatives, but so also are the odourless tannins, while many of the constituents of sweet-smelling essential oils possess an alicyclic structure.

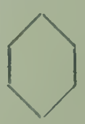
Benzene. Benzene, C_6H_6, is the parent substance of aromatic compounds. None of these contains fewer than six carbon atoms per molecule; and on partial decomposition benzene or one of its derivatives is usually obtained. The molecule of benzene is *symmetrical*, as its monosubstituted derivatives, *e.g.* monochlorobenzene, C_6H_5Cl, do not exist in isomeric forms. Although the molecule is unsaturated, it does not show the instability of unsaturated aliphatic compounds; for instance, benzene does not decolorise bromine water nor an acidified solution of potassium permanganate, nor does it form addition compounds with halogen acids. It does, however, under special conditions combine with two, four or six atoms of halogen or hydrogen per molecule. Two methods of writing the structural formula for benzene are shown in (I) and (II), while (III) is a shorthand notation for the same structure:



(I)

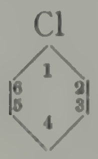


(II)

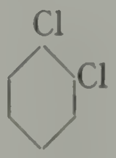


(III)

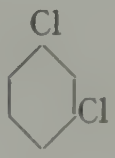
In (III) the substitution of a hydrogen atom is shown by writing the replacing group at the appropriate corner of the hexagon, *e.g.* monochlorobenzene, (IV). In order to facilitate the description of derivatives of benzene, the carbon atoms are usually numbered as in (IV). It will be evident that although only one monosubstituted product is possible, three disubstituted compounds can occur, (V), (VI), and (VII):



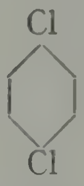
(IV)



(V)



(VI)



(VII)

Formula (V) is 1, 2-dichlorobenzene, or *ortho-*, (*o-*) dichlorobenzene; (VI) is 1, 3-dichlorobenzene, or *meta-*, (*m-*) dichlorobenzene; while (VII) is 1, 4-dichlorobenzene, or *para-*, (*p-*) dichlorobenzene. The univalent radical, C_6H_5, is called the **phenyl** radical.

Benzene itself does not occur in plants, but it can be prepared from benzoic acid, which is yielded by gum benzoin (p. 242). Its technical source is coal tar, one of the products of the dry distillation of coal, which has been produced from plants through geological ages by anaerobic decomposition (partly bacterial), and by the action of high pressures and probably also high temperatures. Benzene is a colourless mobile liquid, with a characteristic odour. It burns with a smoky flame. Being a hydrocarbon, it is a neutral substance. It is immiscible with water, but is one of the best solvents for a large number of organic substances, both liquid and solid.

PHENOLS

The phenols are **hydroxy-derivatives** of benzene and its homologues, in which one or more of the nuclear hydrogen atoms are replaced by hydroxyl groups. The simplest member is **phenol** itself, or carbolic acid, C_6H_5OH. Phenols, by virtue of the hydroxyl group, are *acidic* in character. For instance, they all dissolve in solutions of the caustic alkalis forming metallic salts, *e.g. sodium phenate*, C_6H_5ONa (*cf.* sodium ethoxide, C_2H_5ONa). They do not, however, liberate carbon dioxide from sodium carbonate solutions (contrast carboxylic acids). Most of the phenols give distinctive colour reactions with *ferric chloride* solution, and give *additive* compounds with *bromine*. They also form *ester* and *ether* compounds, and in particular form glycosides with the sugars. Several of the polyhydric phenols occur naturally, and most of the phenols are found among the products of alkaline decomposition of various plant substances such as the tannins, pigments, resins, and wood. The **polyhydric phenols** are colourless, crystalline solids, and differ from the monohydric phenols in being more soluble in water and in exhibiting a reducing reaction, *e.g.* on Fehling's solution. Quinol and pyrogallol are both used as reducing reagents in the form of photographic developers. Again, many of the polyhydric phenols when dissolved in alkalis take up oxygen from the air forming brown or black products. Some of the darkening of plant leaves on injury or death is due to a similar oxidation (p. 110).

Phenol, *carbolic acid,* C_6H_5OH, occurs in coal tar. It is only slightly soluble in water, but is readily soluble in alcohol. When pure it forms colourless needle-shaped crystals, m.p. 42° C.; but in air, owing to its hygroscopicity, it turns reddish-brown in colour, and finally becomes liquid.

EXPT. 58. *Reactions of Phenol*

1. Note the colour developed by the crystals on exposure to air. Show that they are slightly soluble in water.

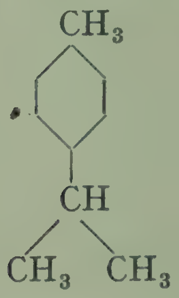
2. Divide the solution into two portions; to (*a*) add ferric chloride solution; a violet coloration is obtained, instantly discharged by dilute hydrochloric acid. To (*b*) add bromine water in excess, when a white precipitate of a tribromophenol separates.

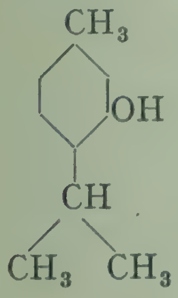
3. Show that crystals of phenol produce no effervescence in sodium carbonate solution.

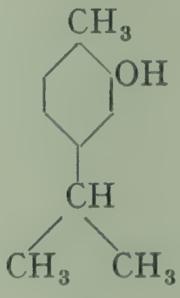
[*N.B.*—Phenol and its concentrated solutions are corrosive and must be handled with care.]

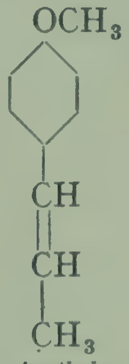
There are three possible monohydroxy-derivatives of *toluene,* $C_6H_5{\cdot}CH_3$, the next higher homologue of benzene, and all three **cresols** (*o*-, *m*-, and *p*-) occur in coal tar. The crude mixture, which also contains phenol, is used under the name 'cresylic acid' as an insecticide and soil-sterilising agent.

Two isomeric phenols, **thymol,** a colourless crystalline substance, and **carvacrol,** an oil, occur in essential oils, especially *oil of thyme* (p. 231). They are hydroxy-derivatives of a hydrocarbon **cymene,** which occurs in many essential oils, including those from several species of *Eucalyptus.* **Anethole,** the principal constituent of *oil of aniseed* and of *fennel oil* (p. 231), is a **phenolic methyl-ether,** containing an unsaturated side-chain.


Cymene

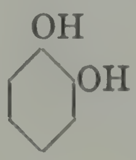

Thymol


Carvacrol

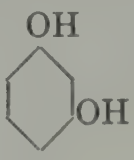

Anethole

Dihydric Phenols

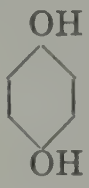
There are three dihydroxy-benzenes, $C_6H_4(OH)_2$, catechol, resorcinol, and quinol (or hydroquinone):



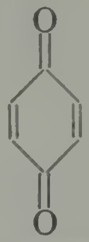
Catechol



Resorcinol



Quinol



Quinone

Catechol, *o*-dihydroxy-benzene, is obtained by the decomposition of certain tannins and resins, especially of the resin *catechu*, obtained from species of *Acacia* indigenous to the East Indies. Catechol also occurs in small amounts in the free state in plants, including species of *Populus* and *Salix*. It is characterised by giving a green colour with ferric chloride, which changes to violet on the addition of sodium carbonate or ammonia.

Resorcinol, *m*-dihydroxy-benzene, is also a decomposition product of various complex substances such as tannins and resins obtained from plants. It gives a deep violet colour with ferric chloride.

Quinol, or hydroquinone, *p*-dihydroxy-benzene, occurs in the free state in the leaves and flowers of the Cranberry (*Vaccinium Oxycoccos*). It also occurs as the monoglucoside **arbutin** (p. 110) in the *Ericaceæ* and leaves of Pear particularly. Ferric chloride solution oxidises quinol to **quinone,** a yellow crystalline substance with a characteristic odour of pepper. Quinone is also obtained by the oxidation of quinic acid from *cinchona bark* (p. 224). Methyl arbutin, which occurs with arbutin, has glucose attached to one of the hydroxyls of quinol, while the other is converted into the methyl ether.

EXPT. 59. *Reactions of Dihydric Phenols*

1. Catechol, resorcinol, and quinol are all soluble in water, giving solutions acid to litmus.

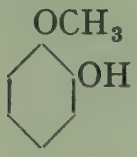
2. All three phenols give precipitates of bromophenols on addition of excess bromine water to their aqueous solutions.

3. To an aqueous solution of the phenol add ferric chloride solution: catechol gives a green colour, turning violet on addition of ammonia; resorcinol gives a violet colour; quinol gives no colour, but on warming the solution, the odour of quinone is noticeable.

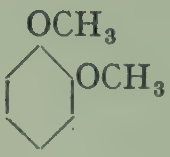
4. Confirm quinol by warming an aqueous solution with manganese dioxide and concentrated sulphuric acid; quinone is formed by oxidation.

The mono- and di-methyl *ethers* of catechol, **guaiacol** and **veratrol** respectively, are also decomposition products of plant substances.

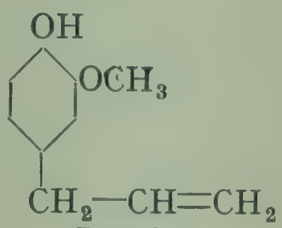
Guaiacol, for instance, occurs in Beechwood tar. **Eugenol,** the main constituent of *oil of cloves* (p. 230), is a monomethyl ether of an *ortho*dihydric phenol with an unsaturated side-chain related to anethole. It also occurs as the glycoside **gein** in *Geum urbanum* (p. 110).



Guaiacol



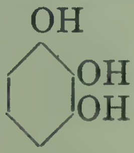
Veratrol



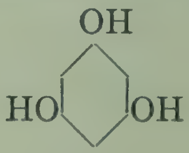
Eugenol

Trihydric Phenols

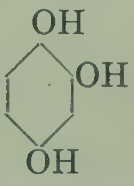
Three trihydroxy-benzenes, $C_6H_3(OH)_3$, are possible, and these are known as pyrogallol, phloroglucinol, and hydroxyquinol.



Pyrogallol



Phloroglucinol



Hydroxyquinol

Pyrogallol, as its name implies, is obtained by heating *gallic acid,* a trihydroxy-acid occurring in gall-nuts (p. 184), carbon dioxide being split off in the reaction. Pyrogallol gives a red colour with ferric chloride solution. In alkaline solution, it absorbs oxygen quantitatively from the air, forming a brown or black solution; it is therefore used for the absorption and estimation of oxygen in gas analysis.

Phloroglucinol is obtained by the alkaline fusion of certain tannins, resins, and anthoxanthin and anthocyanin pigments. It is also found in small amounts uncombined in plants. It gives a violet coloration with ferric chloride, and has a strong reducing action like pyrogallol.

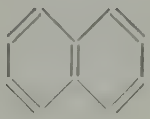
EXPT. 60. *Reactions of Trihydric Phenols*

1. Show that pyrogallol and phloroglucinol are readily soluble in water giving acid solutions.
2. Show that both these solutions absorb bromine from bromine water with the formation of sparingly soluble bromo-derivatives.
3. Show that phloroglucinol gives a violet coloration with ferric chloride solution, while pyrogallol gives a dull red one.
4. Show that both decolorise acidified solutions of potassium permanganate.
5. Dissolve pyrogallol in sodium hydroxide solution and shake with air in a flask. A dark brown colour develops.

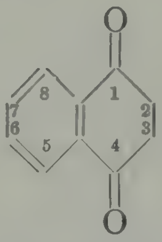
6. Dip a pine shaving or match stick into concentrated hydrochloric acid, then into a solution of phloroglucinol; a red colour is developed (see test 2 on lignin, p. 99).

Naphthalene and Naphthoquinones

Condensed ring systems are possible between benzene nuclei. When two benzene rings are fused together the hydrocarbon **naphthalene** is obtained; it is a product of coal tar distillation. A series of naphthalene derivatives correspond to the benzene derivatives, for instance, the hydroxy-derivatives or **naphthols** correspond to the phenols, and their oxidation products are **naphthoquinones:**



Naphthalene



1, 4-Napthoquinone

Because of the conjugated system of double bonds, the naphthoquinones are coloured substances.

Juglone, $C_{10}H_6O_3$, is isolated as yellow-red crystals from the *Juglandaceæ.* It is 5-hydroxy-1 : 4-naphthoquinone.

Lawsone, $C_{10}H_6O_3$, which is 2-hydroxy-1 : 4-naphthoquinone, occurs in the leaves of an Egyptian shrub, *Lawsonia Inermis*, otherwise known as 'henna'.

The anti-hæmorrhagic **vitamin K_1,** which is widely distributed in the higher plants, is 2-methyl-3-phytyl-1 : 4-naphthoquinone. *Phytyl alcohol*, $C_{20}H_{39}OH$, occurs in chlorophyll and in a variety of other plant compounds; its structure is given on p. 194.

When three benzene rings are condensed, **anthracene** is obtained. The corresponding quinone, **anthraquinone,** has also a conjugated or resonating structure (p. 112), and many of its glycosides were formerly important natural dyestuffs.

Polyhydroxy*cyclo*hexanes. Inositols

Several hydroxy-derivatives of the *reduced* benzene molecule, *hexahydrobenzene*, or preferably *cyclo***hexane**, C_6H_{12}, occur in nature. Such molecules can exist in several isomeric forms, depending on the relative positions of the hydroxyl groups above and below the plane of the ring, as in the sugar molecules. Also, owing to the presence of asymmetric carbon atoms in some of these derivatives, optical isomers are possible.

Quercitol, pentahydroxy-*cyclo*hexane, $C_6H_7(OH)_5$, occurs naturally in two optically active forms, which are not, however, optical antipodes. A *dextro*-form occurs in acorns and in small amounts in the bark of the Oak, also in the leaves of a Palm, *Chamærops humilis*, whereas a *laevo*-form is present in the leaves of *Gymnema sylvestre*.

Inositols. The inositols, hexahydroxy-*cyclo*hexane, $C_6H_6(OH)_6$, are widely distributed in plants. They are isomeric with the monosaccharides ($C_6H_{12}O_6$) and are high-melting, crystalline solids, soluble in water, and possessing a sweet taste. Two optically active forms, an externally compensated form (*dl*-), and a *meso*-form (or forms) have all been found in plants. A *dextro*-modification occurs as the monomethyl ether **pinitol** in the resin of the Californian Pine (*Pinus lambertiana*), and in 'senna' leaves (*Cassia*). A *laevo*-form also occurs as a monomethyl ether, **quebrachitol,** in 'quebrache bark' from *Quebracho colorado* (p. 206), in the latex of *Hevea brasiliensis*, and in the leaves of *Grevillea robusta* and *Heterodendron oleæfolium*. The *meso*-form is the most widespread, its presence having been demonstrated in the seeds of many of the *Leguminosæ*, in leaves of Asparagus, Oak, Ash, and Walnut, in leaves and seeds of the Grape Vine, and in the fruit of the Mistletoe (*Viscum album*). The *dl*-form accompanies the *meso*-form in Mistletoe. Methyl ethers of the optically active and *meso*-forms also occur in the exudations from several Rubber trees. The inositol of animal tissues is always optically inactive.

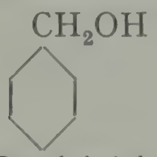
In addition, inositol combines with six molecules of *phosphoric acid* to give the ester-like compound **phytin**; this is present in many, if not all seeds, usually in the form of salts with metals such as calcium and magnesium. Some seeds also contain an enzyme **phytase** which hydrolyses off the phosphoric acid. Phosphorus is intimately connected with the metabolic processes of the plant, and phytin is the principal form of phosphorus storage in seeds, which contain relatively large amounts. It has been suggested that inositol is derived from glucose, and that subsequent dehydration gives the polyhydric phenols, and hence also perhaps more complex molecules such as the anthocyanidins. Inositol is biotin I, a constituent of 'bios', a growth factor required by yeast.

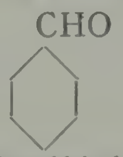
A related compound is **quinic acid,** $C_6H_7(OH)_4COOH$, an optically active modification of which occurs in cinchona bark (p. 224), Coffee beans, and Whortleberries (*Vaccinium Myrtillus*), and is widely distributed in plants as **chlorogenic acid,** its condensation product with *caffeic acid* (p. 184).

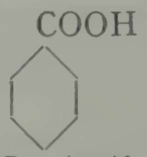
CHAPTER XVIII

AROMATIC ALCOHOLS, ALDEHYDES, AND ACIDS. TANNINS

When a *hydroxyl* group occurs in the side-chain attached to a benzene nucleus, the resulting compound is an **alcohol,** resembling the aliphatic alcohols in many properties. The simplest compound of this type is the primary alcohol, benzyl alcohol, $C_6H_5{\cdot}CH_2OH$. Like the aliphatic alcohols, these alcohols not only form *esters* and *ethers*, but can also be *oxidised* to aldehydes, ketones, and acids; in this, and in their insolubility in alkaline solutions, they are easily distinguished from phenols. Various aromatic alcohols, aldehydes, and carboxylic acids, also their hydroxy-derivatives and the related ethers, occur in plants, sometimes in the free state, sometimes combined as esters in the essential oils or the chemically related balsams and resins, or as glycosides. Their function is obscure, some being probably by-products (but see also p. 113).


Benzyl alcohol


Benzaldehyde


Benzoic acid

Benzyl alcohol is found in several *balsams*, *e.g.* balsams of Tolu and of Peru, and in the *resin storax* (from *Styrax officinalis*), both free and as esters with benzoic and cinnamic acids. Benzyl benzoate is probably formed in the plant by a *Cannizzaro reaction*, in which two molecules of aldehydes undergo self-oxidation and reduction; in this case, one molecule of benzaldehyde is reduced to benzyl alcohol, and simultaneously the second molecule is oxidised to benzoic acid. Similar reactions of aliphatic aldehydes are postulated in various transformations in the plant, *e.g.* in respiration. **Benzyl acetate** is the chief constituent of the essential oil of Jasmine (*Jasminum grandiflorum*).

Benzaldehyde occurs in many of the *cyanophoric glycosides*, *e.g.* amygdalin, prunasin, vicianin, etc. (p. 103).

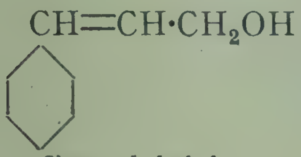
Benzoic acid is present in various *balsams* and *resins*, mainly as esters, *e.g.* benzyl benzoate. Benzoic acid has been known for centuries, its oldest and most important source being *gum benzoin*, which is not a true gum, but a resin from certain species of *Styrax*. Sublimation of the gum yields benzoic acid as colourless, glistening crystals, m.p. 121° C., which can be recrystallised from hot water.

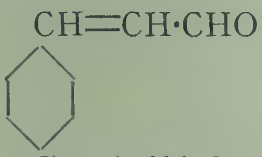
EXPT. 61. *Reactions of Benzoic Acid*

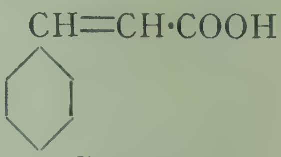
1. Show that benzoic acid is soluble in hot water, and crystallises out on cooling the solution.

2. Show that benzoic acid liberates carbon dioxide with effervescence from warm sodium carbonate solution.

3. Prepare a neutral solution of benzoic acid, and show that on addition of ferric chloride solution a buff-coloured precipitate is formed; and that this dissolves in hydrochloric acid with the separation of a white solid, benzoic acid.

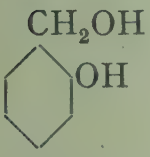

Cinnamyl alcohol

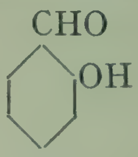

Cinnamic aldehyde

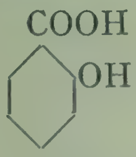

Cinnamic acid

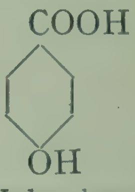
Cinnamyl alcohol is an aromatic alcohol with an unsaturated side-chain. It occurs as an ester with cinnamic acid in *storax*, and is probably formed by a Cannizzaro reaction. **Cinnamic aldehyde** is the main constituent of the essential oils of Cinnamon and Cassia, and like aliphatic aldehydes forms a bisulphite compound (p. 60), which is used in its isolation. **Cinnamic acid** occurs in the form of esters especially with cinnamyl alcohol, in storax and other resins, and in essential oils. The relationship of cinnamic acid to the amino-acid, *phenylalanine*, should be noted, the formula of the latter being derived by saturating the double bond with one molecule of ammonia.

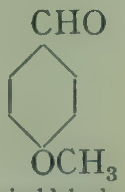
Of the monohydroxy-derivatives of the benzyl alcohol series, the *ortho*-compounds are important natural products.


Saligenin


Salicylaldehyde


Salicylic acid


p-Hydroxybenzoic acid


Anisaldehyde

Saligenin, *salicylic alcohol,* or *o*-hydroxy-benzyl alcohol, is both an aromatic alcohol and a phenol. It is present in the form of its glucoside, **salicin,** in species of Willow and *Spiræa*, and as the more complex benzoyl glucoside, **populin,** in Poplars (p. 111).

Salicylaldehyde, an oily liquid, occurs with the corresponding acid, a colourless, crystalline solid, in the free state in flowers of *Spiræa Ulmaria*; the aldehyde is also present as the glycoside **spiræin** in the roots of some species of *Spiræa*. **Salicylic acid** occurs free in some essential oils, also as **methyl salicylate,** especially in 'oil of wintergreen' from the North American Heath, *Gaultheria* *procumbens*, and in the bark of the Sweet Birch (*Betula lenta*). Glycosides of methyl salicylate include **gaultherin** in the roots of species of *Gaultheria* and *Spiræa*, and **violutin** in *Viola cornuta*.

Expt. 62. *Reactions of Salicylic Acid*

1. Show that salicylic acid is soluble in water giving a solution acid to litmus, and liberating carbon dioxide with effervescence from sodium carbonate.

2. Add ferric chloride solution to a neutral solution of the acid; a purple colour is produced, discharged by hydrochloric acid but not by acetic acid.

3. Heat some crystals of salicylic acid with soda-lime in a test-tube; phenol is formed, as shown by its odour.

4. Warm a mixture of salicylic acid, methyl alcohol, and a few drops of concentrated sulphuric acid in a test-tube; methyl salicylate is formed, producing the odour of 'oil of wintergreen.'

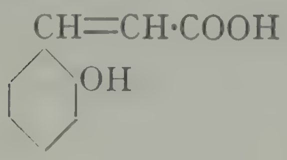
Expt. 63. *Reactions of Salicin*

1. Show that salicin gives a red colour with cold concentrated sulphuric acid.

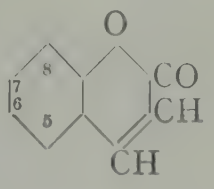
2. Boil a little salicin with dilute sulphuric acid. The salicin is hydrolysed. Cool and filter off the white solid which separates and which is a condensation product of saligenin. Neutralise the filtrate, and show that it contains reducing sugar (glucose) by its action on Fehling's solution.

3. Hydrolysis of salicin with emulsin (expt. on p. 111).

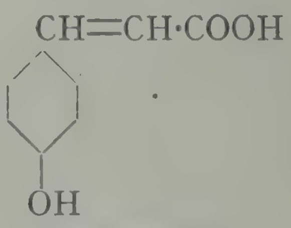
p-**Hydroxy-benzoic acid** occurs combined in the glucosidal pigment **delphinin** (p. 215), while the methyl ether of the corresponding aldehyde is **anisaldehyde,** which occurs in several essential oils, including *oil of anise* (p. 231) and in flowers of the Hawthorn (*Cratægus*).



o-Coumaric acid



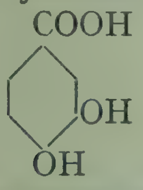
Coumarin

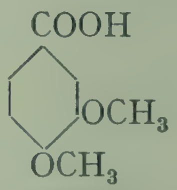


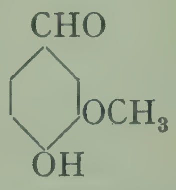
p-Coumaric acid

The corresponding hydroxy-derivatives of cinnamic acid are the coumaric acids. *o*-**Coumaric acid** can form a *lactone* by the loss of a molecule of water between the carboxyl and hydroxyl groups, and the resulting substance **coumarin** is the compound to which new-mown hay and the Woodruff (*Asperula odorata*) owe their fragrance. Coumarin is widely distributed in plants, and is probably present in most of them as glycosides. In addition, the glucoside of *o*-coumaric acid itself, **melilotin,** has been isolated from species of *Melilotus.* A group of glycosides of hydroxy-derivatives of coumarin and of the methyl ethers of such derivatives exist in a large variety of plants, and are usually grouped as the **coumarin glycosides.** Examples are **æsculin** from Horse Chestnut bark (*Æsculus Hippocastanum*), which is the 6-glucoside of 6, 7-dihydroxy-coumarin (æsculetin); **daphnin** from various species of *Daphne,* which is the 7-glucoside of 7, 8-dihydroxy-coumarin (daphnetin); and **fraxin** from species of *Fraxinus,* which is the 8-glucoside of 6-methoxy- 7, 8-dihydroxy-coumarin (fraxetin).

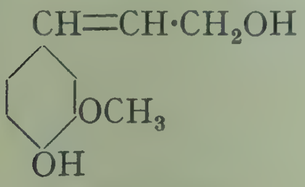
p-**Coumaric acid,** or *p*-hydroxy-cinnamic acid, occurs in the anthocyanins *monardæin* and *gentianin.* It is related to the amino-acid tyrosine in the same way as is *p*-hydroxy-benzoic acid to phenylalanine.

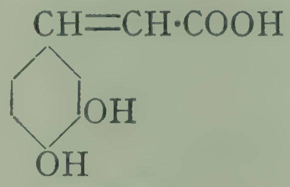

Protocatechuic acid

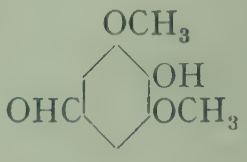

Veratric acid


Vanillin

3, 4-Dihydroxybenzoic acid, or **protocatechuic acid,** occurs in the free state in a few plants, *e.g.* Onion scales, where it appears to be one of the constituents responsible for the resistance of certain varieties to the fungus *Colletotrichum circinans.* It is a constituent of one group of *tannins,* from which it is obtained by dry distillation, and it is also a product of the alkaline decomposition of *resins.* The dimethyl ether of protocatechuic acid, **veratric acid,** has been found in the seeds of *Veratrum sabadilla,* together with an alkaloid veratrine. A related aldehyde is the monohydroxy-monomethyl-ether, **vanillin,** which occurs in vanilla pods, the fruit of the Orchid *Vanilla planifolia*; on heating these, vanillin sublimes as a crystalline solid. It also occurs in some *balsams,* and in traces in the *wood* of many trees. It is prepared artificially by the oxidation of eugenol (p. 177), and may also be obtained from coniferyl alcohol; in both cases the side-chain is ruptured in the oxidation process.

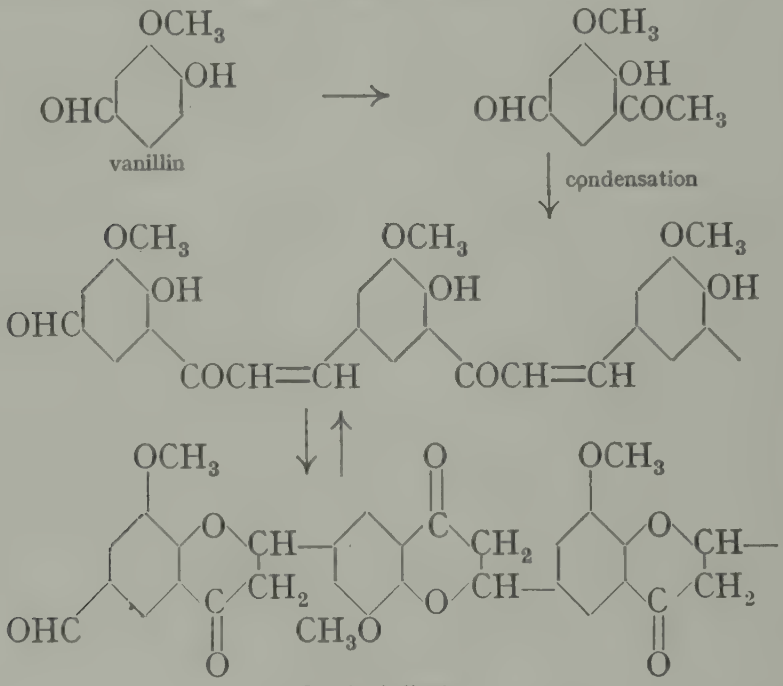

Coniferyl alcohol


Caffeic acid


Syringaldehyde

Of the dihydroxy-derivatives of the cinnamic series, two important natural compounds exist, namely, coniferyl alcohol, which is also a methyl ether, and caffeic acid. **Coniferyl alcohol** occurs, like vanillin, in small amounts in *wood*, and is also a constituent of the glucoside **coniferin** (p. 111). The alcohol can be oxidised to vanillin, and is applied commercially in this way. **Caffeic acid,** or 3, 4-dihydroxy-cinnamic acid, has been isolated from *Clematis vitalba* and *Anthemis nobilis*; it is obtained by the alkaline decomposition of **caffetannic acid,** a tannin occurring in Coffee beans as the calcium and magnesium salts, and in many other plants.

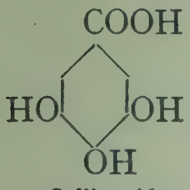
Syringaldehyde is a methoxy-derivative of vanillin, and they occur together as decomposition products of some **lignins** (p. 100). If the structural formulæ are written so that the aldehydic groups are in the position shown, then the relationship of these compounds to each other, and a brief summary of the synthesis by Russell of a lignin comparable to Spruce lignin can be shown:



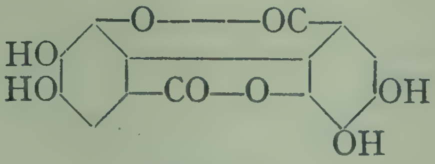
Synthetic lignin

This *dihydrobenzopyrone* nucleus is also present in the *flavanones* (p. 208). Varying lengths of chain are possible, and, as in the flavanones, additional hydroxyl and methoxyl groups. This would explain the difference among lignins, and especially the structure of **secondary lignin** (p. 100), which has a very high molecular weight, and is formed in the wood after the cells are dead.

Gallic acid, or 3, 4, 5-trihydroxy-benzoic acid, occurs in the free state in the bark of several trees, in the woody tissue of some herbaceous plants, in gall-nuts, and in tea. It is probably formed by the hydrolytic decomposition of the tannins, as it always occurs with these, and can be prepared from many tannins by acid hydrolysis. It is a colourless, crystalline substance, readily soluble in water, and having an astringent taste. It gives a blue-black coloration with ferric salts, and is thus used in the manufacture of inks. It resembles pyrogallol, which is obtained by heating gallic acid, in that its alkaline solutions absorb oxygen and darken.



Gallic acid



Ellagic acid

Expt. 64. *Reactions of Gallic Acid*

1. Show that gallic acid gives an aqueous solution which is acid to litmus and which liberates carbon dioxide with effervescence from sodium carbonate.

2. Show that ferric chloride solution gives with gallic acid a blue-black coloration.

3. Show that a solution of gallic acid gives a precipitate with lead acetate, but none with lead nitrate or with gelatine solution (contrast tannin).

Ellagic acid, $C_{14}H_6O_8$, occurs in the free state in plants which contain tannin, *e.g.* Oak bark and Oak galls, and it is probably formed in the plant by the hydrolysis of the tannin. It can also be prepared by boiling an aqueous extract of tannins with dilute sulphuric acid. Structurally, ellagic acid consists of two molecules of gallic acid which have undergone oxidation and condensation. It is owing to the presence of ellagic acid in the tannins concerned that a 'bloom' is produced on leather.

Chlorogenic acid, $C_6H_3(OH)_2CH{:}CH{\cdot}CO{\cdot}O{\cdot}C_6H_7(OH)_3COOH$, is another hydroxy-acid which is very widely distributed in plants. It has been shown by acid hydrolysis to be a condensation product of caffeic (p. 183) and quinic acids (p. 179). Onslow stresses the importance in the oxidative systems in the plant of the many naturally occurring compounds such as chlorogenic acid, catechol, and gallic acid, which contain the *ortho*dihydroxy-grouping, and are therefore oxidisable to a quinone. The oxidation product of chlorogenic acid is, as its name implies, a green pigment. The distribution of chlorogenic acid in plants and its possible function in respiration have been investigated by Oparin.

TANNINS

The tannins form a group of complex substances which are widely distributed in plants. They occur especially in trees and shrubs, and in herbaceous perennials. With a few exceptions, there is little accumulation of tannin in annuals. When tannin is present, it is usually disseminated throughout the whole plant, being to a large extent in solution in the cells; but it accumulates also in the bark of trees and in the more permanent tissues such as underground stems and rootstocks of perennials; and it is found in the dead cells of woody tissue. Tannins are present in certain special structures, such as lactiferous tubes. Many unripe fruits owe their astringent taste to tannins in solution; on ripening, the tannins are either precipitated in an insoluble condition, or are walled off in special cells or *tannin-sacs*, as happens in the ripening of persimmons. This segregation can be artificially induced by treating the fruit with ethylene (p. 316). Tannins accumulate in large amounts in some pathological growths, especially in the galls which are formed by the puncturing of leaves and buds of various species of Oak by the insects *Cynips*, and of Sumach by *Aphis*.

The tannins in the bark, leaves, and wood of a tree are not necessarily all of the same type (*v*. Oak bark and Oak wood tannins). The amount of tannin elaborated by one species may vary with the season, the type of soil, the age of the tissue, etc. In some cases there is accumulation of tannin in the spring, in others in the summer or autumn. Tannins do not appear to perform one definite function in all plants. Various suggestions as to their rôle have been promulgated, *e.g.* they may help, through the antiseptic properties of their simple constituents, in preventing fungal attacks; they may also play some part in pigment formation, as the soluble pigments contain similar phenolic groupings.

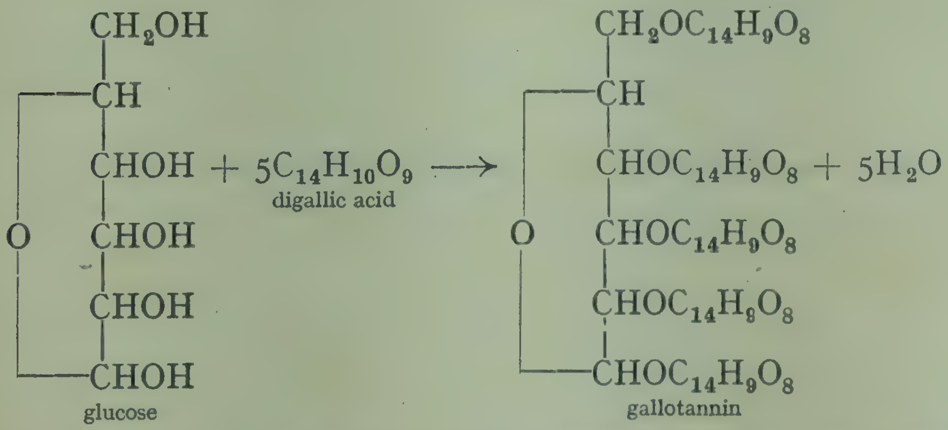
General Properties. Tannins are amorphous substances which form colloidal solutions in water. They have an astringent taste, and give blue-black or green colorations with ferric salts. They precipitate albumin, gelatine, and the alkaloids from solution, and are themselves precipitated by basic lead acetate and lead nitrate. They combine with hide to form leather, the reaction being partly a precipitation of protein similar to the precipitation of gelatine. Extracts from various plant tissues are of commercial importance in the tanning industry, *e.g.* from **galls** (species of *Quercus* and *Rhus*); from **wood** of *Quebracho colorado*, a tree belonging to the *Anacardiaceæ* growing in the Argentine, and of Chestnut (*Castanea vesca*); from **bark** of Hemlock Spruce (*Tsuga canadensis*), and of various species of *Eucalyptus*; from **leaves** of Sumach (*Rhus*); from **fruit** of *Terminalia chebula* in China and the East Indies giving the tannin-extract ‘myrobalans’; from acorn cups of species of *Quercus* from Greece and Asia Minor, giving ‘valonia’; and from pods of species of *Cæsalpinia* in Central America, giving 'divi-divi' and 'algarobilla'. Tannins may be isolated by extraction with water; but mixtures of alcohol, ether, and water, sometimes also with acetone or ethyl acetate, are more commonly used.

Structure. Chemically, the tannins are complex substances of an aromatic nature. Drastic decomposition, as in alkaline fusion, gives various *phenols*, *e.g.* catechol, pyrogallol, and *hydroxy-acids*, *e.g.* gallic and protocatechuic acids. The tannins may perhaps be built up in the plant from these simpler substances by condensation and oxidation reactions. Some of the tannins have been shown by Emil Fischer to be **glucosides,** but it has not yet been demonstrated that all the tannins contain a sugar residue.

The tannins have been divided into two groups depending on the colour given with ferric salts, *viz.* the *iron-bluing* tannins, which were considered to be pyrogallol derivatives, and the *iron-greening* tannins, which were regarded as catechol derivatives. A more exact classification into three groups (Perkin and Everest) is based on the action of boiling dilute sulphuric acid, *viz.* (*a*) tannins which give gallic acid (Gallotannins or Depsides); (*b*) tannins which give ellagic acid (Ellagitannins); (*c*) tannins which give an amorphous red-coloured precipitate called 'phlobaphene' (Phlobotannins or Catechol Tannins). The phlobotannins are also distinguished by giving a yellow or brown precipitate with bromine water. Many commerical tannin extracts are mixtures, and contain members of more than one group.

Gallotannins. The most important tannin of this group goes under the various names *gallotannin*, *tannic acid*, *gallotannic acid*,

$$\underset{\text{gallic acid}}{2C_7H_6O_5} \longrightarrow \underset{\text{digallic acid}}{C_{14}H_{10}O_9} + H_2O$$



and occurs in large quantities in galls, especially of the Oak, *Quercus infectoria* (50–60 per cent. in Aleppo galls), and the Sumach, *Rhus* *semilata* (70 per cent. in Chinese galls). It also occurs in various plants, and is the main tannin in the commercial extracts of Sumach, of *Cæsalpinia*, and 'valonia'. The commercial gallotannic acid is an amorphous, yellow or brown substance, which is readily soluble in water to give a slightly acid solution. It is also soluble in alcohol, but insoluble in ether. It gives a blue-black coloration or precipitate with ferric chloride. When dissolved in alkali it absorbs oxygen and the solution darkens (*cf.* pyrogallol and gallic acid), and on boiling this solution *gallic acid* is obtained. Fischer showed that gallotannin was optically active, and on hydrolysis gave *glucose* as well as gallic acid. He considered it to be a compound of one molecule of glucose with five molecules of a digallic acid, and prepared several synthetic compounds of this type which exhibited the properties of tannins.

EXPT. 65. *Reactions of Gallotannin*

To separate portions of an aqueous solution of commercial tannic acid add (*a*) ferric chloride solution (a blue-black coloration or precipitate is produced); (*b*) lead acetate solution (the tannin is precipitated); (*c*) lead nitrate solution (the tannin is again precipitated); (*d*) gelatine solution (the gelatine is precipitated).

The tannin from Chestnut wood (*Castanea*) is similar to gallotannin, and so is that from the bark of *Hamamelis virginiana*. This last differs from most tannins in that it has been isolated in a crystalline form, and contains a sugar with a branched chain (p. 73).

Ellagitannins. Pomegranate tannin from the root bark of *Punica granatum* is a glucoside of ellagic acid.

Phlobotannins. These tannins usually give a green colour with ferric chloride, and on alkaline decomposition yield *catechol* or *protocatechuic acid* and sometimes phloroglucinol and resorcinol as well. The last two products may be due to a different part of the molecule from that responsible for the phlobaphene reaction. The presence of sugar in the molecule is doubtful in all members of this group; possibly, however, the constitution may be similar to that of the other groups, except that several different phenolic residues may be attached to the sugar residue in the molecule.

A number of *bark* tannins belong to this group, *viz.* Willow bark (*Salix triandra*), Larch bark (*Larix europæa*), Cherry bark (*Prunus Cerasus*), cortepinitannic acid from Scotch Fir bark (*Pinus sylvestris*), Hemlock Spruce bark (*Tsuga canadensis*), maletto tannin from the bark of *Eucalyptus occidentalis*, and quercitannic acid from Oak bark (*Quercus*). This last, which gives a green colour with ferric chloride, must not be confused with the tannin from Oak wood, which gives a blue colour, and is probably a gallotannin. **Caffetannic acid** in the form of its calcium and magnesium salts occurs in Coffee beans (*Coffea arabica*), in Paraguay tea (*Ilex paraguensis*), in *Strychnos Nux-vomica*, and in St. Ignatius' beans (*Strychnos Ignatii*). The molecule contains glucose, and on decomposition with various reagents yields caffeic acid, catechol, and protocatechuic acid. It gives a green coloration with ferric chloride solution.

EXPT. 66. *Extraction of Tannins*

Pound some Oak galls, or cut up Oak bark or Sweet Chesnut bark, and boil it with a little water. Filter and test the filtrate, as above, for tannic acid. These should all give blue colorations with ferric chloride.

Repeat with Horse Chestnut bark, or Walnut bark; these will give a green colour with ferric chloride.

CHAPTER XIX

PLANT PIGMENTS

One of the most striking features of the plant world is its variety of **colour.** Pigments and dyes from plants were used by primitive man, but the isolation of the actual pigments and the elucidation of their structure is one of the triumphs of modern chemistry.

The pigments in plants can be divided into two groups depending on where and how they occur; firstly the **plastid** pigments, and secondly the **cell-sap** or soluble pigments. The former comprise two types of pigments, the **chlorophylls,** which give the green colour to plants, and the **carotenoids,** which are responsible for many of the yellow and orange hues in nature. They occur in specialised structures, the plastids. They are insoluble in water, but soluble in fats and in fat-solvents. The cell-sap pigments, which are soluble in water, occur in the cell-sap. Here there are also two groups, the **anthoxanthins** and the **anthocyanins.** The anthocyanins give the red, purple, and blue colour to flowers, and they are also present in most leaves. The anthoxanthins, when they occur alone, rarely colour flowers, but their presence modifies all other colours, especially those of the anthocyanins. Many flower colours, especially bronze shades, are due to the combined effect of both plastid and cell-sap pigments.

Autumn and Winter Colouring. In green leaves the presence of chlorophyll masks the other pigments, but in deciduous trees in the autumn the chlorophyll is destroyed, probably by oxidative reactions, while the carotenoids persist. The amount of carotene decreases as the leaves fade, but the total carotenoid content for autumn and green leaves is approximately the same. The yellow autumn foliage of Poplars, Hazel, Walnut, and Chestnut is due to carotenoids. In most cases, however, anthocyanin, which is always present in small amounts in the leaves, increases in quantity, and the autumn leaves of most trees, including the Hawthorn, the European Maple (*Acer campestris*) and members of the *Rosaceæ*, owe their colour to both carotenoids and anthocyanins. The red winter foliage of plants which retain their leaves is also mostly due to anthocyanins, *e.g.* in leaves of the Saxifrage and Privet; but in a few cases carotenoids alone are responsible, namely in the Yew and other *Coniferæ*. Colour changes during the ripening of fruit may involve one or both groups of pigments; *e.g.* in the

Banana the carotenoids alone are responsible for the yellow colour of the ripe fruit.

PLASTID PIGMENTS

In the higher plants there exist specialised structures of microscopic size termed **plastids,** composed of a colourless substance, the **stroma,** which appears to be mainly protein. Some plastids contain chlorophyll, and these, the so-called **chloroplasts,** give the green colour to leaves and stems, and are the seat of the photosynthetic process. The chloroplasts also contain several carotenoids, especially the carotenes and xanthophylls. Other plastids, called **chromoplasts,** contain no chlorophyll, but are coloured yellow, orange, or even red, by the presence of carotenoid pigments. These carotenoids are present in the plastids in a granular and sometimes even in a crystalline form, and they give the colour to most yellow and orange flowers, to some fruits and seeds, and to some naturally yellow leaves. An exception to the occurrence of the carotenoids in the plastids is found in several members of the *Ranunculaceæ,* in which the yellow flowers have an oily appearance, *e.g.* the Buttercups and Marsh Marigolds; here, the colour is due to the carotenoids being dissolved in oil in the cells.

Separation of the Chloroplast Pigments. As early as 1864, Stokes succeeded in showing that the pigments of the green leaf consisted of at least four components, two green and two yellow. This work, which was achieved by extracting the mixed pigments and separating them by partition between immiscible solvents, was disregarded by later investigators. The isolation of two yellow pigments was repeated by several workers, but it was not till 1907–1913 that Willstätter's brilliant researches on leaf pigments showed that there were two chlorophylls, and that one of the yellow pigments was identical with carotene, the pigment of Carrots. Willstätter also showed that the proportions of these various pigments in green leaves remained remarkably constant. The ratio of chlorophylls to carotenoids was approximately three to one, although in shade leaves this ratio was sometimes increased. The ratio of chlorophyll *a* to chlorophyll *b* was very constant, being about three to one, while there was about twice as much xanthophyll as carotene. That there were not just two yellow pigments was shown by Tswett's chromatograph (1910); by filtering a solution of the mixture of chloroplast pigments through a column of tightly packed, dry calcium carbonate, he found that the various pigments were adsorbed at different rates, yielding a banded structure, which indicated the presence of about five yellow pigments. The more recent work of Karrer and Kuhn has established that there are several carotenes and several xanthophylls, and that in certain plants there are other substances chemically related to these, the whole group being described as the carotenoids.

The following experiments demonstrate some of the methods employed by Willstätter in the separation of the pigments:—

Expt. 67. *Extraction of the Chloroplast Pigments*

Soak 4 grm. of dried Nettle leaves with about 50 c.c. of 90 per cent. acetone; then filter. Pour the deep blue-green solution, which contains all the pigments, into twice its volume of ether in a separatory funnel. Then pour a little distilled water down the side of the funnel, and gently rotate the funnel, but do not shake. Run off the aqueous layer, and repeat this washing several times to remove all acetone from the ether solution.

Expt. 68. *Removal of Chlorophylls* a *and* b

Now run in 10 c.c. of a 30 per cent. solution of potassium hydroxide in methyl alcohol, and note that the colour changes to a yellowish-brown, and on shaking gradually changes back to green (the Phase Test). On addition of water, a green colour appears in the aqueous layer, and the ethereal layer is yellow, since it now contains only the carotenoids. The green aqueous layer contains the *chlorophyllins*, the two chlorophylls having undergone saponification and oxidation. Run off this alkaline layer into another separatory funnel, and add an excess of concentrated hydrochloric acid. The magnesium is split off and a blue solution is formed which can be transferred to ether by shaking after dilution, giving a brownish-green solution of *chlorins* and *rhodins*.

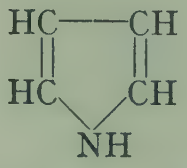
Expt. 69. *Separation of the Carotene and Xanthophyll*

The ethereal solution of the carotenoids from the above experiment is washed twice with water by shaking in a separatory funnel, and is then evaporated almost to dryness in an evaporating dish on a hot water-bath with no flame. The residue is taken up in about 20 c.c. of petroleum ether, and shaken with an equal volume of 90 per cent. methyl alcohol. This extracts the xanthophylls, while the carotene remains in the petroleum ether.

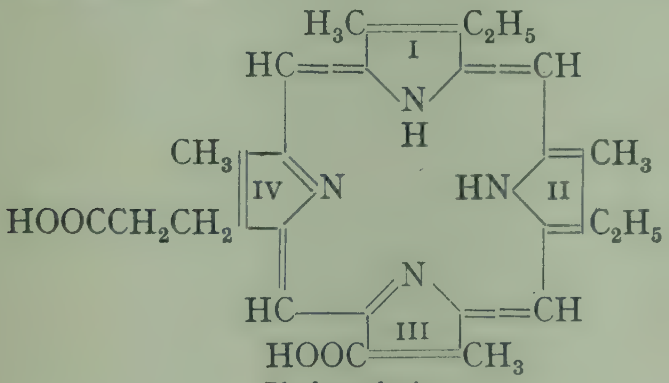
The Chlorophylls

Chlorophyll is developed in plants soon after the plumule reaches the light, and light seems necessary for its formation, although a very low intensity is sufficient in some cases, as seedlings of Pines develop chlorophyll while they are still within the cotyledons. In etiolated shoots and leaves, chlorophyll formation is inhibited; but some colourless precursor with a structure resembling that of chlorophyll must be present, as such tissues turn green very rapidly on exposure to light.

Structure. Willstätter's researches showed the chemical composition and the fundamental structure of the chlorophyll molecule. Chlorophyll contains *carbon, hydrogen, oxygen, nitrogen,* and *magnesium* in the molecule, and on drastic degradation yields **pyrroles,** heterocyclic substances with the following basic five-membered ring structure:—



Less drastic degradation by alkali fusion gives **porphyrins,** compounds containing four pyrrole rings arranged in a symmetrical fashion. *Hæmin,* the coloured part of the *hæmoglobin* molecule, resembles chlorophyll in that the molecule contains a metal, in this case iron, and on similar degradation also yields pyrroles and porphyrins. Similar metal-porphyrin-protein complexes occur in some of the plant enzymes (p. 198). Hans Fischer achieved a brilliant synthesis of hæmin, and he also synthesised several of the porphyrins derived from chlorophyll, the chief one being **rhodoporphyrin,** $C_{32}H_{34}N_4O_4$. The latter is a substituted porphyrin



Rhodoporphyrin

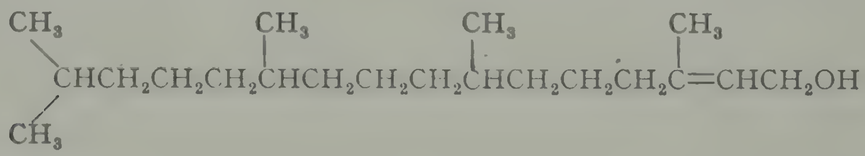
with the above formula. Willstätter showed that chlorophyll, when carefully treated with oxalic acid, loses magnesium, and yields a waxy substance called **phæophytin.** This has no acid properties, and therefore the magnesium must be attached to *nitrogen,* and cannot be present as a salt of an acid. Hydrolysis of phæophytin with hot alkali gives **methyl alcohol,** an unsaturated alcohol **phytol,** and an acidic material containing thirty-four carbon atoms per molecule. The method of *acid fractionation,* namely, extraction from ether solution with hydrochloric acid solutions of different strengths, and returning the extracted material to ether by dilution with water and shaking with fresh ether, shows that there are two acidic products: one of these gives a green ether solution and is called **phytochlorin** *e* (usually shortened to **chlorin** *e*), and the other gives a reddish-brown ether solution and is called **phytorhodin** or **rhodin** *g*. The names chlorin and rhodin are used for any chlorophyll derivative with these respective colours, and letters are attached to designate the various substances of this character obtained by different reactions. Chlorophyll, then, consists of two compounds, designated *a* and *b*, the former giving rise to chlorin *e*, the latter to rhodin *g*. Chlorophyll *b* has two hydrogen atoms less, and one oxygen atom more, than chlorophyll *a*, and this oxygen atom is present as a formyl group, (-CHO), which persists through many reactions. The two chlorophylls differ in solubility and in the colour and absorption spectra of their solutions; chlorophyll *a* is more soluble in petroleum ether, chlorophyll *b* in methyl alcohol. The stability of this formyl group, and the constant proportions in which the two chlorophylls occur in green leaves, preclude any possibility of the reversible oxidation of chlorophyll *a* to *b* in the photosynthetic process.

Expt. 70. *Separation of Chlorophyll* a *and* b

Transfer an acetone extract from 2 grm. of dried nettle leaves to light petroleum in an analogous fashion to the preparation of the ether solution (p. 192). After washing the light petroleum solution with water, shake it with 90 per cent. methyl alcohol. The alcohol layer is green, and contains chlorophyll *b* and some xanthophyll, while the light petroleum layer is blue-green and contains chlorophyll *a* and carotene.

The phase test with cold alcoholic alkali on chlorophyll *a* can be shown directly with the light petroleum solution; chlorophyll *b* must first be transferred to ether by dilution with water of the methyl alcohol solution in the presence of fresh ether, the aqueous layer removed, and the alcoholic alkali added to the ether layer.

The alcohol **phytol,** $C_{20}H_{39}OH$, occurs in both of the chlorophylls, and has been shown by synthesis to have the following formula:—



$$(CH_3)_2CHCH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2CH(CH_3)CH_2CH_2CH_2C(CH_3){=}CHCH_2OH$$

It is therefore a branched-chain, unsaturated, monohydric primary alcohol. It consists of reduced *isoprene* units (p. 239) and is structurally related both to the essential oils and to the carotenoids.

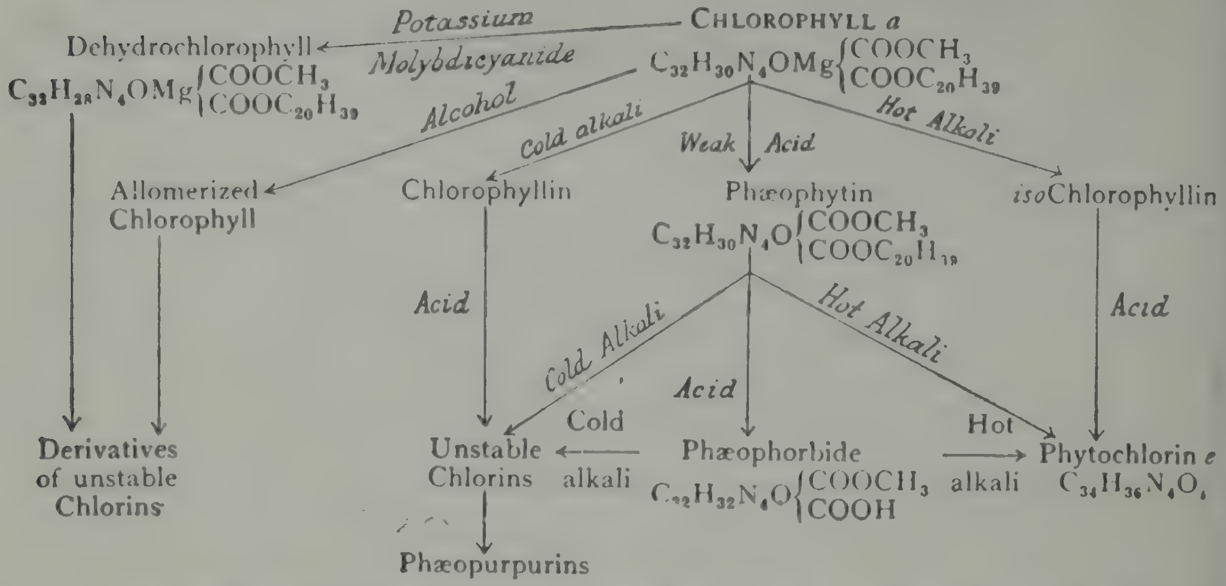
It was early recognised by botanists that if sections of the green leaves of some plants were soaked in alcohol, large crystals were developed in the chloroplasts, and the substance concerned was called **crystalline chlorophyll.** Willstätter showed that these plants, *e.g.* Hogweed (*Heracleum Sphondylium*), Hemp-Nettle (*Galeopsis Tetrahit*), Hedge Woundwort (*Stachys sylvatica*), contain an enzyme **chlorophyllase** which hydrolyses the phytol group and replaces it by the alcohol present, *e.g.* methyl alcohol.

Reactions of Chlorophyll *a*. Chlorophyll *a* is more easily obtained pure than chlorophyll *b*, and the following reactions were done first with chlorophyll *a*, or with phæophytin *a* (the separation of the phæophytins being easier than that of the chlorophylls), but parallel results are given by chlorophyll *b*.

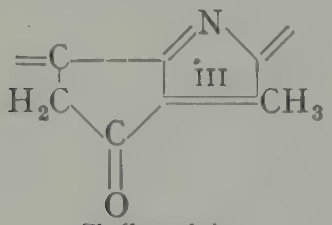
The hydrolysis of phæophytin *a* to chlorin *e* can take place in two stages; concentrated hydrochloric acid removes only the phytol, giving an acidic substance **phæophorbide** *a*, and this on treatment with hot alkali also gives chlorin *e*. This same compound can be obtained by treating chlorophyll *a* first with hot alcoholic alkali, which removes the phytol and gives *iso***chlorophyllin,** and then with acid, which removes the magnesium.

A completely different set of products is obtained if any one of chlorophyll, phæophytin, or phæophorbide *a* is treated with cold alcoholic alkali. The ethereal solution of chlorophyll *a* on being shaken with the alkali turns first yellow then finally green, and hence the reaction is known as the **phase test.** (Similarly chlorophyll *b* gives a brown-red, then a green solution, and a mixture of *a* and *b* gives a brown intermediate colour.) The product from chlorophyll *a* is **chlorophyllin,** not identical with *iso*chlorophyllin; on treatment with acid it gives **chlorins** which are different from chlorin *e*, and these, when kept in ether solution, give compounds which are purplish-red in colour, the **phæopurpurins.** Similarly the phase test gives these 'unstable' chlorins when applied to phæophytin or to phæophorbide *a*.

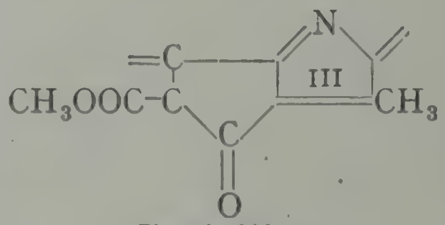
If chlorophyll *a* is kept in alcoholic solution a new substance is obtained, termed by Willstätter **allomerised chlorophyll,** which no longer gives the phase test. Conant has shown that removal of the magnesium and phytol from allomerised chlorophyll gives products of the unstable chlorin series and not of the chlorin *e* type. Hence allomerization and the phase test are comparable reactions, and have been shown to be **oxidations** with atmospheric oxygen. Chlorophyll *a* can also be oxidised by potassium molybdicyanide to **dehydrochlorophyll,** which again, on removal of magnesium and phytol, gives products of the unstable chlorin series. Hence chlorophyll *a* readily undergoes oxidation by dehydrogenation, according to the equation, $RH_2 + O \longrightarrow R + H_2O$. The following diagram summarises the reactions described above:—


CHLOROPHYLL a
$C_{32}H_{30}N_4OMg\begin{cases}COOCH_3\\COOC_{20}H_{39}\end{cases}$
Potassium
Molybdicyanide
Dehydrochlorophyll
$C_{33}H_{28}N_4OMg\begin{cases}COOCH_3\\COOC_{20}H_{39}\end{cases}$
Alcohol
Cold alkali
Weak
Acid
Hot Alkali
Allomerized Chlorophyll
Chlorophyllin
Phæophytin
$C_{32}H_{30}N_4O\begin{cases}COOCH_3\\COOC_{20}H_{39}\end{cases}$
isoChlorophyllin
Acid
Cold Alkali
Hot Alkali
Acid
Acid
Cold
alkali
Hot
alkali
Derivatives of unstable Chlorins
Unstable Chlorins
Phæophorbide
$C_{32}H_{32}N_4O\begin{cases}COOCH_3\\COOH\end{cases}$
Phytochlorin e
$C_{34}H_{36}N_4O_6$
Phæopurpurins

Further degradation of chlorin *e* results in the formation of rhodoporphyrin. Conant showed that the propionic acid group (on ring IV) was esterified with phytol in the original chlorophyll molecule. Meanwhile Hans Fischer used a mild hydrogen-iodide degradation on phæophorbide, and obtained a series of porphyrins which were more complex than the rhodoporphyrin above; they contained a *five-membered carbocyclic ring* (between the carboxyl group on ring III and the adjacent methylene bridge), which the reactions showed existed also in chlorophyll. **Phylloerythrin,** which belongs to this group of compounds, is a biological decomposition product of chlorophyll in herbivorous animals. It has the following partial formula:

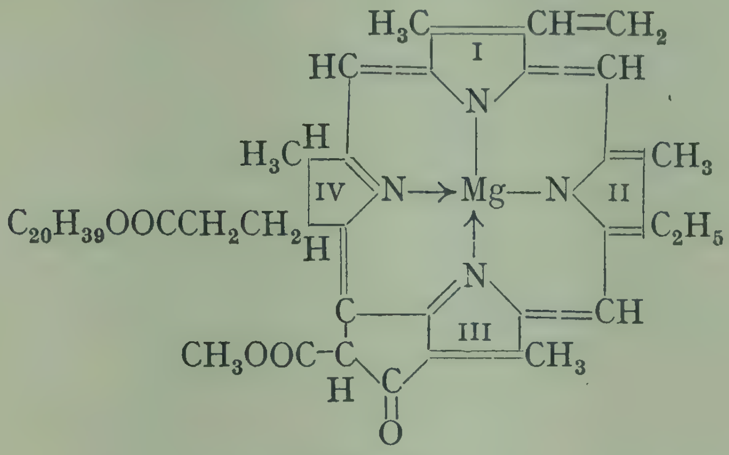


Phylloerythrin



Phæophorbide *a*

Phæophorbide *a* contains in addition a *carbomethoxyl* group on this five-membered ring. Phæophorbide, however, is not a porphyrin, but an isomer. Fischer showed that chlorophyll, the phorbides, and the chlorins all contained (i) a *vinyl group* ($-CH=CH_2$) in place of the ethyl group on ring I, and (ii) a *dihydroporphyrin nucleus.* Porphyrin formation with hydrogen iodide is a shift of these 'extra hydrogen atoms' to the vinyl group, which is thereby saturated to the ethyl group of rhodoporphyrin. The formation of the chlorins, on the other hand, leaves the vinyl group intact, and opens and decomposes the carbocycylic ring, forming the carboxyl group on ring III. The position of the 'extra hydrogen atoms' has not been finally established; ring IV, as shown in the formula for chlorophyll *a* is their most likely position.



Chlorophyll *a*

In **chlorophyll** *b*, the methyl group on ring II of chlorophyll *a* is replaced by the formyl group (–CHO). **Bacteriochlorophyll,** the assimilatory pigment of the photosynthetic purple and brown bacteria, contains a dihydrophorbide (or tetrahydroporphyrin) nucleus, and the vinyl group of chlorophyll *a* (ring I) is replaced by an acetyl group ($-COCH_3$). In some plants an apparent precursor of chlorophyll, *viz.* **protochlorophyll,** is found. It is the porphyrin of chlorophyll *a*; that is, it does not contain the two 'extra hydrogen atoms'.

Chlorophyll in the Chloroplasts. Absolute alcohol or pure acetone will not extract chlorophyll from dried leaves, but the chlorophyll is easily removed when from 5 to 10 per cent. of water is present. Hence chlorophyll occurs in the chloroplasts bound to the stroma in such a way that the stability of the colloidal system of the plastid must be disturbed before extraction is possible. A definite conjugated protein, **chloroplastin** has been isolated (p. 148). The function of chlorophyll in photosynthesis is discussed in a later chapter (pp. 265 and 269).

IRON PORPHYRINS

The **hæmin** (or hæme) of **hæmoglobin** is a ferric iron porphyrin, and when conjugated with the protein globin forms a system which adds on oxygen and releases it to the cell. Similar compounds of iron porphyrins and proteins occur in all living cells. Hæmoglobin itself occurs in the root nodules of *Leguminosæ* which contain the nitrogen-fixing bacteria, *Rhizobium*. Neither the plant roots nor the bacteria separately are able to synthesise hæmoglobin, but their symbiosis leads to its formation. The chemical relationship with the fixation of nitrogen must be one of oxygen transfer.

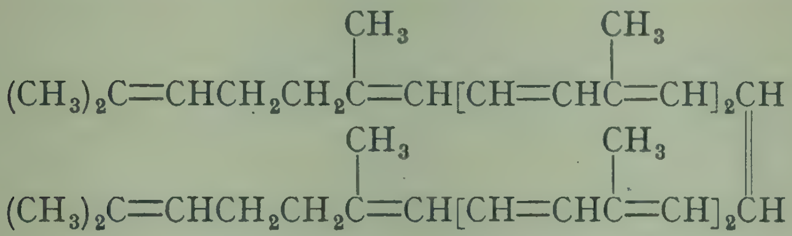
The corresponding *ferrous* iron porphyrin (ferrous protoporphyrin IX) is the iron porphyrin most widely distributed in plants. It has been detected in minute amounts in various plant tissues, *viz.* roots, seeds, and leaves, and it is also present in the enzyme **catalase,** where it is conjugated with protein. (Protoporphyrin IX is related to rhodoporphyrin as follows: the ethyl groups on rings I and II of rhodoporphyrin are replaced by vinyl groups, and the carboxyl group on ring III is another propionic acid group.) **Coproporphyrin** I, which is present in cereal grains and in yeast, is an iron tetramethyltetrapropionyl-porphyrin. **Peroxidase** and the **cytochromes** are also iron porphyrins. All these enzymes are concerned with oxygen transfer, which will be discussed under respiration (p. 284). The mechanism of the **synthesis** of **porphyrins** in the plant has been indicated by the use of a nutrient solution containing *glycine* labelled with isotopic (heavy) nitrogen. This was found in the porphyrin compounds synthesised by the plant. *In vitro*, Fischer showed that pyrrole formation was possible under mild conditions from the condensation of glycine and formyl-acetone.

The Carotenoids

Carotenoids occur in both plants and animals; in the latter they are derived from, although they are not always identical with, the plant carotenoids in the food. They are all soluble in the 'fat-solvents' and remain in the unsaponifiable residue; the term *lipochrome* is therefore sometimes applied to them. The carotenoids of plants belong to either the **hydrocarbons** or to **oxygen-containing compounds**; the latter group is subdivided into (*a*) substances containing hydroxyl, aldehyde, or keto groups, and (*b*) those containing carboxyl groups. They all contain unsaturated linkages arranged in a special order ('conjugated' double bonds) which confers on them their peculiar colour, their distinctive absorption spectra, and the instability of the hydrocarbons and group (*a*) in the presence of oxygen. They may all be regarded as composed of **isoprene** nuclei, the structural unit of the terpenes, and are closely related to phytol, from which it has been suggested that some of them, *e.g.* lycopene, are derived.

Hydrocarbon Carotenoids. There are three widely distributed hydrocarbon carotenoids. They are isomeric, conforming to the formula $C_{40}H_{56}$, and consist of two **carotenes,** α- and β-, and **lycopene.**

Lycopene is the pigment of the fruit of the Tomato (*Solanum Lycopersicum*); it also occurs in other fruits, often with carotene and xanthophyll, *e.g.* in species of *Berberis* and *Rosa*, in some evergreen leaves, and in the scales on the cones of some Conifers. Lycopene forms red crystals, giving yellow solutions in ether and alcohol, and a bluish-red solution in carbon disulphide. Duggar has shown that lycopene is formed in tomatoes only when these are ripened under 30° C.; green tomatoes ripened above 30° C. turn yellow owing to the development of other carotenoids, including carotene, but lycopene will still develop if the temperature is lowered to 20° or 25° C. Lycopene has thirteen double bonds per molecule, and can be hydrogenated to give a saturated hydrocarbon, $C_{40}H_{82}$; hence lycopene is an *aliphatic* or open-chain hydrocarbon, and has the following symmetrical formula:—

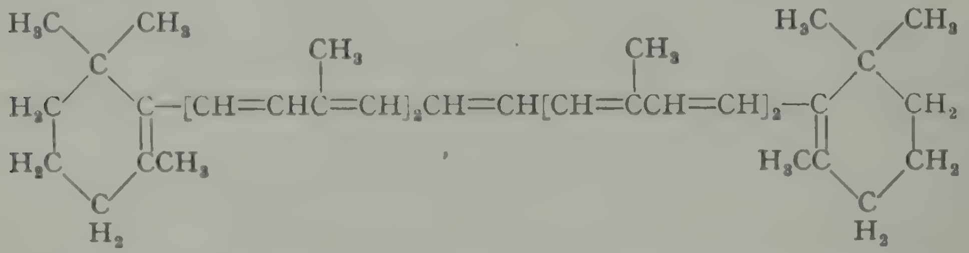


$$\begin{array}{r}
(\mathrm{CH_3})_2\mathrm{C{=}CHCH_2CH_2}\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{C}}}\mathrm{{=}CH[CH{=}CH}\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{C}}}\mathrm{{=}CH]_2CH} \\
\| \\
(\mathrm{CH_3})_2\mathrm{C{=}CHCH_2CH_2}\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{C}}}\mathrm{{=}CH[CH{=}CH}\overset{\mathrm{CH_3}}{\overset{|}{\mathrm{C}}}\mathrm{{=}CH]_2CH}
\end{array}$$

This molecule is equivalent to two molecules of phytol condensed together and subjected to loss of hydrogen to give the unsaturation.

Carotene, $C_{40}H_{56}$, was the first carotenoid isolated from plants, its source being the root of the Carrot (*Daucus Carota*). It consists of red crystals, insoluble in alcohol, but very soluble in benzene and in carbon disulphide to give in the latter case a blood-red colour. The carrot pigment is also present in the chloroplasts of green leaves, and it gives the colour to many flowers and some fruits. For instance, crystals of carotene have been isolated from the Daffodil, from the corona of the Narcissus, and from various members of the *Ranunculaceæ*. Xanthophylls are usually present in smaller amounts along with carotene. Several isomeric carotenes occur in plants. Two forms, α- and β-, are common, but have a different distribution, β-carotene being more widespread. For instance, grass, Spinach, and stinging Nettles contain only β-carotene, whereas Carrots contain 10–20 per cent. of α-carotene, and palm oil 30–50 per cent. of α-carotene, the remainder being the β-form. α-Carotene has a melting-point of 172° C. and is *dextro*-rotatory, whereas β-carotene melts at 183°–184° C. and is symmetrical and therefore optically inactive. All the carotenes contain eleven double bonds, and the isomerism is probably due to the arrangement of these in the molecule. Unlike lycopene, the carotene molecule has

—in addition to an *unsaturated aliphatic* part—two ring structures, one at each end of the chain. These are identical with the **β-ionone ring** (compare the irone ring, p. 234) in the terpenes, and the whole molecule of carotene is built up of isoprene units. The symmetrical molecule of β-carotene can be explained by the following structure:—



The effect of the carotenoids on animal nutrition has undergone a great deal of investigation, because of the relationship of these substances to vitamin A. It has been shown that the carotenes and kryptoxanthin (from *Physalis*), but none of the other purified carotenoids, can be converted into the vitamin in the animal organism. The carotene molecule undergoes oxidative fission with the formation of several substances, including vitamin A, $C_{20}H_{30}O$. This has been synthesised; its formula is identical with that of half of the carotene molecule, the terminal carbon atom at the point of fission being present as a primary alcoholic group. Since the vitamin molecule still contains unsaturated linkages like the parent carotene, its function is probably—to some extent at least—that of an oxidising catalyst. Evidence has been adduced that there are in plants several carotenoids containing, like carotene, forty carbon atoms in the molecule, which are split by oxidation into a mixture of products, some of which are the other carotenoid pigments of smaller molecular weight isolated from plants, *e.g.* bixin.

Alcohol Carotenoids. The usual group name for the carotenoids of plants containing alcoholic hydroxyl groups is **xanthophylls.** These include the xanthophylls of the chloroplasts, and some of them occur in higher concentrations in certain plants.

The monohydroxycarotenes, $C_{40}H_{55}OH$, include **lycoxanthin** in the Tomato and Deadly Nightshade (*Solanum Dulcamara*), **kryptoxanthin** in the berries of *Physalis* species, and in yellow Maize, and **rubixanthin** in hips (especially of *Rosa Rubiginosa*) and haws.

The dihydroxycarotenes, $C_{40}H_{54}(OH)_2$, include **lycophyll** in the Tomato and Deadly Nightshade, and **lutein** in grass, Spinach, Nettle, Clover, and many yellow flowers (it is also one of the pigments of egg-yolk). **Zeaxanthin** occurs in the seeds of Maize, in the seeds and husks of *Euonymus Europæus*, and in *Physalis*.

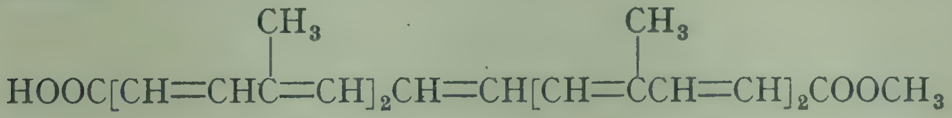
Trihydroxycarotene, $C_{40}H_{53}(OH)_3$, is the structure of **flavoxanthin**, the yellowest of the xanthophylls. It occurs in the Buttercup (*Ranunculus Acer*) and in Groundsel (*Senecio Vernalis*).

The tetrahydroxycarotenes, $C_{40}H_{52}(OH)_4$, include **violaxanthin** in the petals of the yellow Pansy (*Viola tricolor*), and **taraxanthin** in the Dandelion (*Taraxacum officinale*), in Coltsfoot (*Tussilago Farfara*), and in Touch-me-not (*Impatiens*).

These alcohols are often present in the plant as **esters,** especially of fatty acids. For instance, the pigment of the Winter Cherry (*Physalis*) is **physalien,** the dipalmitic ester of zeaxanthin, and **helenien,** the flower pigment of *Helenium autumnale*, is the dipalmitic ester of lutein.

Ketone and Aldehyde Carotenoids. Rhodoxanthin, $C_{40}H_{50}O_2$, is a diketone isolated from the calyx of the ripe seed of the Yew (*Taxus Baccata*), and from the leaves of species of Juniper Cypress. Other carotenoids contain both hydroxyl and carbonyl groups: **capsanthin,** $C_{40}H_{38}O_3$, is a dihydroxy-ketone isolated from the skin of the ripe fruit of Spanish Pepper (*Capsicum annuum*), while **fucoxanthin,** $C_{40}H_{60}O_6$, occurs in some brown Seaweeds and in some of the *Phæophyceæ*.

Carboxylic Acid Carotenoids. The carboxylic acid class contains the only carotenoids stable to oxygen. The following have been isolated: **crocetin,** $C_{20}H_{24}O_4$, the pigment of saffron, the dried stigma of *Crocus sativus*; **bixin,** $C_{25}H_{30}O_4$, from *Bixa orellana*; and **azaffrin** from the South American plant *Escobedia*. Crocetin is an exceptional instance of a carotenoid occurring in the plant as a glycoside, *viz.* **crocin,** the digentiobioside. Crocetin has certain hormone effects on the sexual reproduction of some green algæ, and appears to be similar to, if not identical with, the naturally secreted hormone. Bixin is the monomethyl ester of a dicarboxylic acid with the following symmetrical structure. It will be seen that this is similar to the central portion of the lycopene and carotene molecules:



$$\mathrm{HOOC[CH{=}CH\overset{\overset{CH_3}{|}}{C}{=}CH]_2CH{=}CH[CH{=}\overset{\overset{CH_3}{|}}{C}CH{=}CH]_2COOCH_3}$$

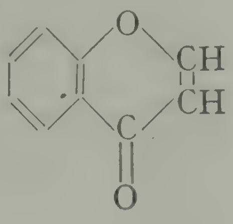
EXPT. 71. *Carotenoids in Flowers*

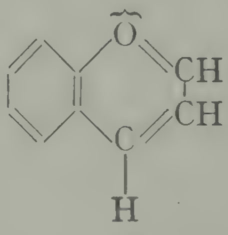
Steep some yellow flower-heads such as Sunflower (*Helianthus annuus*), *Escholtzia*, or Dandelion, in 95 per cent. alcohol. To one portion of the resulting yellow extract add concentrated hydrochloric acid; a green or blue-green colour is obtained. Shake the other portion in a separatory funnel with an equal volume of light petroleum; carotene will be extracted from the alcohol and colour the petroleum solution yellow, while the xanthophylls, if present, will remain in the alcohol layer. Run off the alcohol layer, and evaporate the petroleum solution to dryness on a water-bath with no flame. Show that the residue dissolves in carbon disulphide to give a red solution.

Physiological Function. Apart from their biological function in flowers and fruits, of attracting insects for pollination and birds and animals to ensure the spreading of the seed, the function of the carotenoids in plant metabolism is not clear. Various suggestions have been advanced: (*a*) By absorbing certain light rays, they may act as *energy transformers*; but as the main absorption bands of the carotenoids are in the blue and violet, little heat energy can be obtained. (*b*) By such absorption they may *protect the cell enzymes* against harmful light rays of short wave-length. (*c*) The carotenoids, as we have seen, absorb oxygen readily, hence they may function as *oxygen carriers*, or oxidising catalysts in plants; there is no justification, however, for the view, promulgated before the chemical nature of the carotenoids was known, that carotene is reversibly oxidised to xanthophyll in the photosynthetic process. The *synthesis* of the carotenoids in plants appears to be independent of light, but *dependent on temperature*; it has already been noted that lycopene is only developed at relatively low temperatures.

The Cell-Sap Pigments

These pigments belong to two groups, the **anthoxanthins** and the **anthocyanins,** which have a related *heterocyclic* structure. The former contain the **benzopyrone** nucleus (I) and the latter the **benzopyrylium** structure (II):—


(I)


(II)

They all contain phenolic hydroxyl groups which form coloured salts with alkalis. All the anthocyanins are **glycosides**; many of the anthoxanthins are also combined in the plant as glycosides, but some also occur in the free state.

The Anthoxanthins

General Properties. The glycosides of the anthoxanthins are practically colourless, and therefore give little colour to plant tissues. Their presence can be readily demonstrated in most white flowers by the development of a yellow colour on treatment with alkali. The free anthoxanthins are yellow in colour, as are their potassium salts. A few yellow flower colours are due to these soluble pigments, for instance, Indian Cotton (*Gossypium herbaceum*) and yellow *Antirrhinum*. The anthoxanthins and their glycosides are characterised by the *yellow* or reddish-yellow solutions they give with *alkalis*; if glycosides are used the colour is discharged by acidification. They also give dull green or reddish-brown colours with ferric chloride solution, and are precipitated by lead acetate solution (a method often employed in their isolation) giving yellow or yellowish-red precipitates. Before the widespread use of synthetic dyestuffs, metallic salts of the anthoxanthins were used extensively as dyes, the cloth being first 'mordanted' with compounds of iron, aluminium, and other metals, and then treated with the plant extracts, various shades of green, brown, and yellow being obtained.

Expt. 72. *Detection of Anthoxanthins*

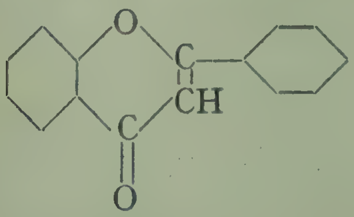
1. Place white flower heads or petals in a flask with a few drops of concentrated ammonia; a yellow colour is produced in a few minutes. (Suitable flowers are Narcissus, Snowdrop, white Stock, white Phlox, white Chrysanthemum.)

2. Extract white flowers with water or aqueous alcohol on a water-bath, decant the solution, and divide it into three portions. Add, (*a*) alkali (a yellow colour is produced), then acidify (the colour is discharged); (*b*) lead acetate solution (a yellow or orange precipitate of the lead salt is obtained); (*c*) ferric chloride solution (a green or brown colour is produced).

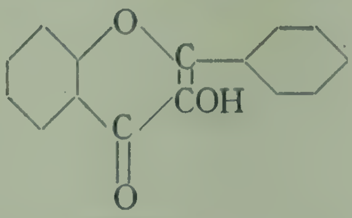
Similar tests may be made on aqueous extracts of green leaves, especially of Parsley.

Structure. The structure of the anthoxanthins has been determined by the examination of their decomposition products, which include phenols, and phenolic acids such as protocatechuic acid. Their formulæ have in many cases been verified by synthesis. The investigators who have contributed most to the elucidation of anthoxanthin structure are Arthur Perkin, and Kostanecki; and more recently Robinson has evolved methods of synthesis of the glycosides themselves.

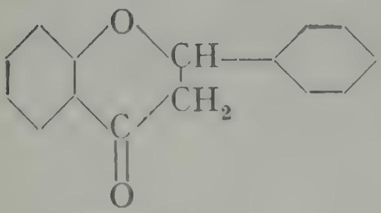
The anthoxanthins are hydroxy-derivatives of the following five types, **flavone, flavonol, flavanone,** *iso***flavone,** and **xanthone**:—



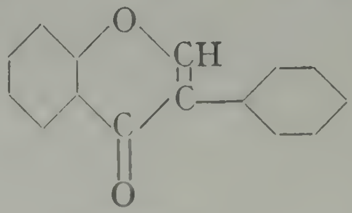
Flavone



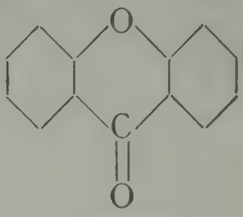
Flavonol



Flavanone



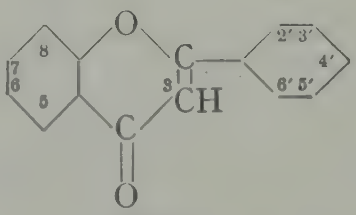
*iso*Flavone



Xanthone

It will be seen that xanthones differ from the others in containing a benzene nucleus fused on to the benzopyrone nucleus; that is, they are dibenzopyrones, whereas in the other four types the benzene nucleus replaces a hydrogen atom in the benzopyrone nucleus. Flavones and *iso*flavones differ in the position of this attachment. Flavonols contain a hydroxy-pyrone nucleus, and flavanones contain a dihydro-pyrone nucleus. The compounds isolated from plants are *hydroxy-derivatives* of these fundamental compounds, the hydroxyl groups being attached both to the benzopyrone nucleus and to the single benzene nucleus. Some of the natural compounds are also *methyl ethers*, and most of them are glycosides. In some instances, although the pigment may exist as a glycoside in the plant, it cannot be isolated without using conditions which would possibly admit of hydrolysis. Various sugars are found, including some di- and tri-saccharides peculiar to these pigments; so far, no case has been found of two sugar groups being attached to different hydroxyl groups (contrast the anthocyanins), nor has the sugar been found in combination with the uncondensed benzene ring. Isomerism in the anthoxanthins is conditioned both by differences in the position of the hydroxyl groups, and by the point of attachment of the sugar residue.

Flavones and Flavone Glycosides. The flavones are hydroxy-derivatives of flavone itself, usually combined with a sugar to form glycosides. In two cases, both from *Scutellaria*, the flavones are combined with glucuronic acid in place of glucose. The most important members of the flavone group are chrysin, apigenin, and luteolin.



Flavone

Flavone itself, $C_{15}H_{10}O_2$, is found as the characteristic dust or 'farina' on the flower-stalks, leaves, and seed capsules of many varieties of *Primula*, especially *P. pulverulenta* and *P. japonica*.

It has been suggested that its repellant action towards water, caused by a change in the surface tension, may be of physiological use to the plant.

Chrysin, $C_{15}H_{10}O_4$, is 5, 7-dihydroxy-flavone. It occurs in the leaf buds of several species of Poplar (*Populus*) and also in Mallows (*Malva*). Associated with chrysin in Poplar buds is its 7-monomethyl ether, **tectochrysin.**

Apigenin, $C_{15}H_{10}O_5$, is 5, 7, 4′-trihydroxy-flavone. It occurs chiefly as the diglycoside **apiin**; this is present in the leaves, stems, and seeds of Parsley (*Petroselinum sativum*), from which it can be extracted with boiling water. Hydrolysis of apiin, $C_{26}H_{28}O_{14}$, with very dilute acid gives an apigenin glucoside and a sugar called *apiose*, $C_5H_{10}O_5$; and on more drastic treatment with acid the glucoside breaks down to give apigenin and glucose. Apiose, which has not been found in any other plant, is one of the rare sugars containing a branched chain (p. 73). The disaccharide, glucoapiose, is attached to a flavone hydroxyl (probably in position 7) through the glucose nucleus. A glucoside of apigenin occurs in Chamomile flowers (*Anthemis nobilis*), and is hydrolysed by dilute acid to glucose and apigenin. Another derivative of apigenin is **acaciin,** occurring in the leaves of the common or false Acacia (*Robinia pseud-acacia*). It is a dirhamnoside of the 4′-methyl ether of apigenin, **acacetin,** the disaccharide being probably attached to the 7-hydroxyl group.

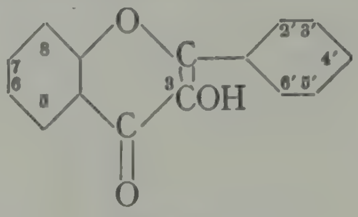
The 5, 6, 7-trihydroxy-flavone, baicalein, occurs in **baicalin** in the roots of *Scutellaria baicalensis*, combined with glucuronic acid.

Luteolin, $C_{15}H_{10}O_6$, which is 5, 7, 3′, 4′-tetrahydroxy-flavone, is the main pigment of Dyer's Weld (*Reseda luteola*); it is also present in Dyer's Broom (*Genista tinctoria*), *Digitalis purpurea*, and in *Antirrhinum* flowers. **Galuteolin** is a glucoside of luteolin obtained from the seeds of *Galega officinalis*. 4′-Methyl-luteolin, or diosmetin, occurs as the 7-rhamnoglucoside, **diosmin** in *Scrophularia nodosa*, and several other plants. Diosmetin is also present as the 7-apiose glycoside along with apiin in the stems and leaves of Parsley, but not in the seeds.

The 5, 6, 7, 4′-tetrahydroxyl-flavone, scutellarien, is obtained along with glucuronic acid by the hydrolysis of **scutellarin** occurring in the leaves of *Scutellaria baicalensis*, and the flowers of *S. altissima*.

Lotoflavin is 5, 7, 2′, 4′-tetrahydroxy-flavone; it occurs as the cyanophoric glycoside **lotusin** in the leguminous plant *Lotus arabicus* of Northern Africa. On hydrolysis, lotusin yields lotoflavin, gentiobiose, and hydrocyanic acid.

Flavonols and their Glycosides. Flavonol itself is 3-hydroxy-flavone, and its most important hydroxy-derivatives are galangin, fisetin, kaempferol, quercetin, morin, and myricetin; these occur free or as glycosides. The flavonols differ from the flavones in that their alkaline solutions are readily oxidised by air.



Flavonol

Galangin, 5, 7-dihydroxy-flavanol, occurs as a glycoside with the 3-methyl ether of galangin in 'Galanga root,' the rhizomes of *Alpinia officinarum.*

Fisetin, 7, 3′, 4′-trihydroxy-flavonol, occurs both uncombined and as a glycoside, **fustin,** in 'young fustic,' the wood of the stem and larger branches of *Rhus cotinus*; fisetin and fustin are found also in the wood of *Quebracho colorado*. Fustin on hydrolysis yields fisetin and rhamnose.

Kaempferol, 5, 7, 4′-trihydroxy-flavonol, has been isolated from the flowers of *Delphinium consolida* and *D. zalil* (the Indian dye 'asbarg'), from flowers of *Prunus spinosa*, and from the berries of the Buckthorn (*Rhamnus catharticus*). **Kaempferide,** the 4′-methyl ether of kaempferol, occurs in 'Galanga root'. A similar and perhaps identical methyl ether occurs in the berries of *Rhamnus catharticus*. At least four glycosides of kaempferol are known. A monorhamnoside is the main constituent of the efflorescence found on several Australian species of *Acacia*. **Kaempferitrin,** the 3-dirhamnoside, occurs in the leaves of one of the Indigo plants, *Indigofera arrecta*; while **kaempferin,** the 3-diglucoside, occurs in 'senna' leaves (from various species of *Cassia*). **Robinin,** which occurs in the flowers of *Robinia pseud-acacia*, gives on hydrolysis kaempferol and a trisaccharide, *robinose* (p. 78).

Datiscetin, 5, 7, 2′-trihydroxy-flavonol, occurs in the root and leaves of the Bastard Hemp (*Datisca cannabina*) as **datiscin,** a glycoside giving the disaccharide *rutinose* on hydrolysis (p. 75).

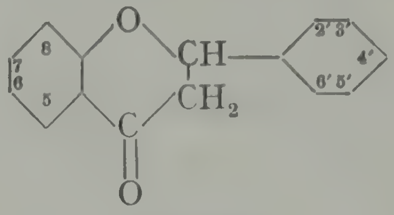
Quercetin, 5, 7, 3′, 4′-tetrahydroxy-flavonol, one of the most widely distributed anthoxanthins, has been isolated both in the free state and as glycosides from a variety of plant tissues. Five monoglycosides are known. **Quercitrin,** a 3-rhamnoside of quercetin, occurs in the inner bark ('quercitron bark') of the Bark Oak (*Quercus discolor* or *tinctoria*), a native of America. **Incarnatrin** is a glucoside occurring in the Crimson Clover (*Trifolium incar-*

natum). *iso***Quercitrin** and **quercimeritrin** occur in the flowers of some species of Cotton (*Gossypium*), the former being quercetin 3-glucoside, and the latter the 7-glucoside. Another monoglucoside is **serotin,** which occurs in the wild Black Cherry (*Prunus serotina*). A single diglycoside, **rutin,** has been isolated from a number of plants, including leaves of Rue (*Ruta graveolens*), 'capers' (from *Capparis spinosa*), Buckwheat (*Fagopyrum esculentum*), and buds of a Chinese tree of the *Leguminosæ* (*Sophora japonica*) used for dyeing purposes. The sugar, *rutinose,* is attached to the 3-position. Several methyl ethers of quercetin and their glycosides have also been isolated. Rhamnetin, the 7-monomethyl ether, occurs as the glycoside **xanthorhamnin** from 'Persian berries' (*Rhamnus*); this glycoside contains in the 3-position the trisaccharide *rhamninose* (p. 78), an isomer of robinose. *iso*-**Rhamnetin,** the 3′-monomethyl ether of quercetin, occurs in the Wallflower (*Cheiranthus Cheiri*), in the Red Clover (*Trifolium pratense*), and in 'senna' leaves, and as a glucoside in the flowers of *Delphinium zalil.* **Rhamnazin,** a 7, 3′-dimethyl ether, also occurs in 'Persian berries.' Various other plants in which quercetin occurs either free or as glycosides include Ling (*Calluna vulgaris*), the skins of the Onion bulb (*Allium Cepa*), and the flowers of Horse Chestnut (*Æsculus Hippocastanum*), Hawthorn (*Cratægus Oxyacantha*), Fuchsia (*Fuchsia macrostema globosa*), and the Clovers (*Trifolium pratense, T. incarnatum,* and *T. repens*).

Morin, 5, 7, 2′, 4′-tetrahydroxy-flavonol, an isomer of quercetin, occurs in 'old fustic,' the wood of the tree *Chlorophora tinctoria* (formerly *Morus tinctoria*), which grows in the tropics, especially in the West Indies. 'Old fustic' also contains a pentahydroxybenzophenone, **maclurin,** and is still of importance as a dyestuff. Morin and maclurin are present in the wood of the Osage Orange tree (*Maclura aurantiaca*), a native of America, and morin occurs in 'Jak-wood' obtained from the Indian tree, *Artocarpus integrifolia* of the *Urticaceæ.*

Several pentahydroxy-flavonols and their glycosides are known. **Myricetin,** 5, 7, 3′, 4′, 5′-pentahydroxy-flavonol, occurs free and combined with rhamnose in **myricitrin** in the bark of the Box Myrtle (*Myrica rubra* or *nagi*), in leaves of the Sicilian Sumach (*Rhus coriaria*) and Venetian Sumach (*R. cotinus*), and in leaves of the Logwood tree (*Hæmatoxylon campechianum*). Gossypetin, 5, 7, 8, 3′, 4′-pentahydroxy-flavonol, occurs as the monoglucoside **gossypitrin** in various species of Cotton (*Gossypium*), while **quercetagetin,** 5, 6, 7, 3′, 4′-pentahydroxy-flavonol, occurs as a monoglucoside in flowers of the African Marigold (*Tagetes patula*).

Flavanones and their Glycosides. The structure of this group of pigments has only been elucidated in recent years. They are hydroxy-derivatives of flavanone, that is flavone in which the double bond in the pyrone nucleus has been reduced.



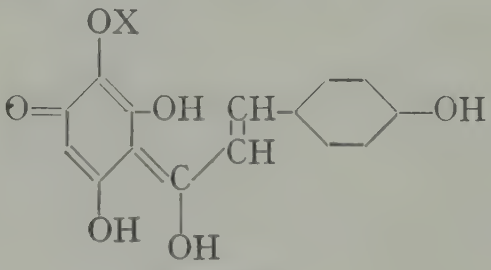
Flavanone

The following are some of the members of this group:—

Naringenin, 5, 7, 4′-trihydroxy-flavanone, occurs as the glycoside **naringin** in the flowers of *Citrus decumana.* The sugar is rhamnose or possibly a rhamnoglucose. The 7-monomethyl ether of naringenin, sakuranetin, occurs as the glucoside **sakuranin** in the bark of several Japanese species of *Prunus.* **Butin,** 7, 3′, 4′-trihydroxy-flavanone, is present as a glucoside in the flowers of the Indian Dhak tree (*Butea frondosa*) of the *Leguminosæ.*

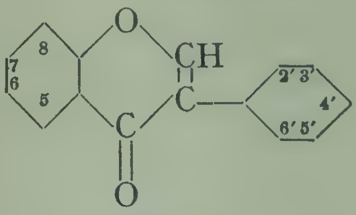
Hesperitin is the 4′-monomethyl ether of 5, 7, 3′, 4′-tetrahydroxy-flavanone. It occurs in the peel of most *Citrus* fruits including the Orange (*Citrus Aurantium*) and the Lemon (*C. Limonum*) as **hesperidin,** the 7-rhamnoglucoside.

Carthamin is a glucoside obtained from the Safflower or Bastard Saffron (*Carthamus tinctorius*), an annual of the *Cynarocephalæ* cultivated in Europe and the East for its dyeing properties. On hydrolysis it yields glucose, carthamidin, or 5, 7, 8, 4′-tetrahydroxy-flavanone, and *iso*carthamidin, or 5, 6, 7, 4′-tetrahydroxy-flavanone. Carthamin itself does not contain these two compounds, but consists of two six-membered rings joined by an unsaturated chain, termed a *chalkone* structure, and on hydrolysis of the glucose (X) from the 8-position, ring closure takes place.



Carthamin

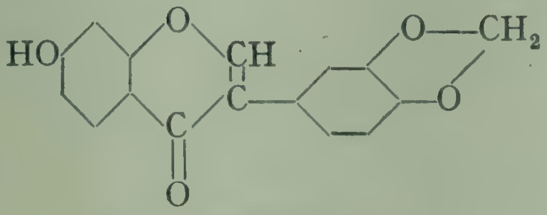
***iso*Flavones and their Glycosides.** As *iso*flavone differs from flavone only in the position of the single benzene nucleus, the properties of their derivatives are very similar. The following *iso*flavones are characteristic of the group:



iso-Flavone

Genistein, 5, 7, 4′-trihydroxy-*iso*flavone, occurs with luteolin as the pigment of the Dyer's Broom (*Genista tinctoria*), and as the 7-glucoside in the Soya Bean. The 4′-methyl ether of genistein occurs as the glucoside **prunetin** in the bark of species of *Prunus*.

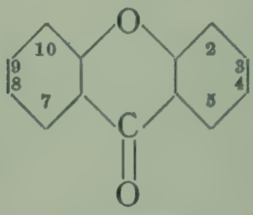
7, 3′, 4′-Trihydroxy-*iso*flavone forms a methylene derivative with the adjacent hydroxyl groups, and this compound, ψ-baptigenin, occurs as the rhamnoglucoside **ψ-baptisin** in the roots of *Baptisia tinctoria*.



ψ-Baptigenin

Irigenin is the 6, 4′, 5′-trimethyl ether of 5, 6, 7, 3′, 4′, 5′-hexahydroxy-*iso*flavone, and occurs condensed with glucose in position 7 as **iridin** in 'Florentine iris root,' the dried rhizomes of *Iris florentina*, *I. germanica*, and *I. pallida*.

Xanthones. Several pigments have been diagnosed as hydroxy-derivatives of dibenzopyrone or xanthone.



Xanthone

Euxanthone, 4, 7-dihydroxy-xanthone, occurs as the pigment 'Indian yellow,' consisting of the magnesium and calcium salts of euxanthic acid, in the urine of cattle fed on Mango leaves (*Mangifera indica*). On hydrolysis euxanthone is obtained, also glucuronic acid, which is attached to the hydroxyl in position 4.

Gentisin, the pigment of the Gentian root (especially *Gentiana lutea*) is the 9-methyl ether of gentisein, or 4, 7, 9-trihydroxyxanthone.

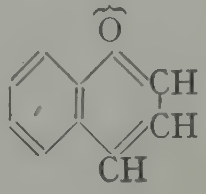
The Anthocyanins

The anthocyanins are the red, violet, and blue pigments which occur in solution in the cell-sap of flowers, many fruits, and some stems and leaves. In leaves, according to Onslow, they are especially present on the underside in marsh and forest plants, *e.g.* several of the Saxifrages (*S. Geum* and *S. sarmentosa*) and Water Lilies (*Nymphæa*), also in tropical and subtropical plants cultivated for their coloured leaves, *e.g. Tradescantia discolor.* The anthocyanins are also to a large extent responsible for autumn colourings of leaves, and the similar tinting of young shoots and buds in the spring. When anthocyanin is a normal constituent of mature tissues the pigment is found in the epidermis; but when it predominates only temporarily, as in spring and autumn foliage, it is mostly in the mesophyll.

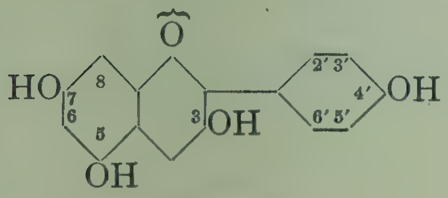
Structure. The anthocyanins are all *glycosides*, the most common sugars being glucose, galactose, and rhamnose. When more than one molecule of monosaccharide is present, these may be attached to different positions in the molecule (contrast the known anthoxanthins) to give **dimonoglycosides,** or they may be attached to one position to form a disaccharide derivative, *e.g.* a **diglycoside.** Most of the anthocyanins fall into definite glycoside groups: *viz.* the 3-*glucosides* and 3-*galactosides* (see formula on p. 211 for numbering); the 3-*pentoseglycosides*, including 3-rhamnoglucosides; the 3-*diglucosides*, and the 3, 5-*dimonoglucosides*. The 3, 5-dimonoglucosides are the most widely distributed of the anthocyanins. They are distinguished by their reaction to alkali in that they give blue solutions with aqueous sodium carbonate solution, fading to yellow on addition of dilute sodium hydroxide; the other glycoside types give violet solutions with sodium carbonate, turning blue with alkali.

Hydrolysis of the anthocyanins gives the sugar or sugars, an **anthocyanidin,** and sometimes other residues, including *p*-hydroxybenzoic acid and *p*-hydroxy-cinnamic acid. These acids are usually attached to the sugar residue by condensation with a hydroxyl group (*acyl* derivatives), *e.g.* in cyanin; in salvianin, however, the hydroxy-cinnamic acid appears to be attached directly to the anthocyanidin part of the molecule.

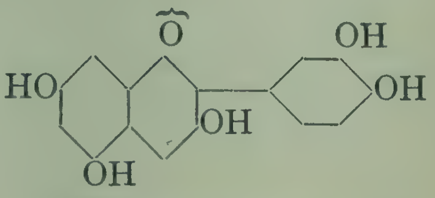
Willstätter first showed that the anthocyanidins contained the **benzopyrylium** nucleus:—



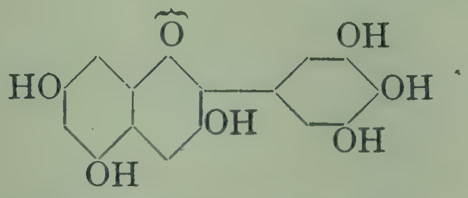
Since the oxygen atom has three valencies concerned in ring formation it is *quadrivalent*, the fourth valency forming stable **oxonium salts,** such as chlorides and acetates, when isolated from the appropriate acid solution. These oxonium salts are used extensively in the isolation of both the anthocyanins and anthocyanidins. Willstätter found that upon alkaline fusion the anthocyanidins break down to give phloroglucinol and phenolic acids (*cf.* the anthoxanthins). So far, the naturally occurring anthocyanins (with the exceptions noted on p. 213) are glycosides of the following *three* anthocyanidins and their methyl ethers:—



Pelargonidin



Cyanidin
Peonidin = cyanidin 3′-methyl ether

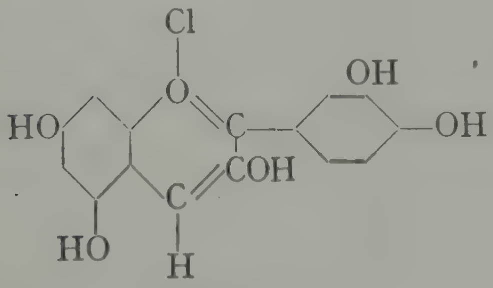


Delphinidin
Petunidin = delphinidin 3′-methyl ether
Malvidin = ,, 3′, 5′-dimethyl ether
Hirsutidin = ,, 7, 3′ 5′-trimethyl ether

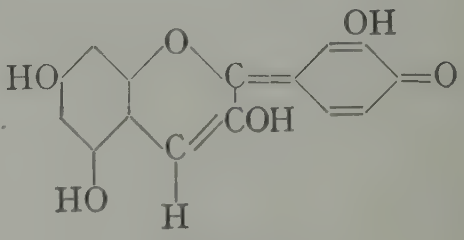
The whole phenyl-benzopyrylium structure is sometimes termed the **flavylium** nucleus. On fusion with alkali, pelargonidin gives *p*-hydroxy-benzoic acid, cyanidin gives protocatechuic acid, and delphinidin gives gallic acid. The structures of all these anthocyanidins and also of some of the anthocyanins themselves have been confirmed by synthesis, largely by the methods of Robinson.

It is obvious from the preceding formulæ that a close structural relationship exists between the anthoxanthins and the anthocyanidins; several of the latter have been synthesised in small amounts from the flavonol pigments by reduction. In the majority of flowers, however, the anthoxanthins and anthocyanidins which occur together are not chemically related in this simple way; even when they are, the glycosides in which they occur often have the sugar residues in different positions. It is more probable that both the anthoxanthins and anthocyanins are synthesised independently from a common and simpler source.

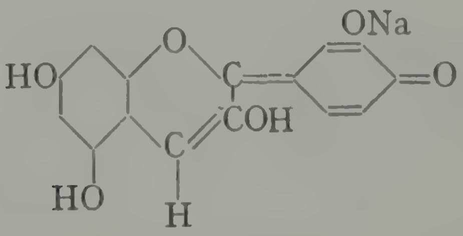
Properties. The anthocyanins and anthocyanidins are amphoteric substances, forming salts with acids by virtue of the oxygen atom, and salts with bases by virtue of the phenolic hydroxyl groups. Their solutions give three distinct colours at different p_H values corresponding to three modifications of the molecule, *viz.* red in acid, violet in neutral, and blue in alkaline solution correspond respectively to an *oxonium salt*, a *colour-base*, and an *alkali salt* of the colour-base. These three forms of cyanidin, using hydrochloric acid and sodium hydroxide as the acid and base respectively, are shown below:—



oxonium salt (red)
$p_H = 3$



colour base (violet)
$p_H = 8{\cdot}5$



alkali salt (blue)
$p_H = 11$

In addition, a colourless or *leuco*-form is obtained on evaporating an anthocyanidin solution to dryness or on allowing a dilute solution to stand in air. The oxonium salt is, however, regenerated on addition of acid. The anthocyanins are usually extracted from the plant tissues with water containing dilute acetic or hydrochloric acids, giving the corresponding oxonium salt of the glycoside. Hydrolysis to the anthocyanidin is effected by warming with hydrochloric acid, when the oxonium salt of the anthocyanidin itself is obtained:

$$\underset{\text{chrysanthemin chloride}}{C_{21}H_{21}O_{11}Cl} + H_2O \longrightarrow \underset{\text{cyanidin chloride}}{C_{15}H_{11}O_6Cl} + \underset{\text{glucose}}{C_6H_{12}O_6}$$

The anthocyanins are soluble in water and fairly soluble in alcohol, whereas the anthocyanidins are less soluble in water and more soluble in organic solvents such as amyl alcohol. This solubility distinction also holds among the different types of glycosides: the diglycosides remain in the aqueous layer when shaken with amyl alcohol; the anthocyanidins are completely extracted by the alcohol; while the monoglycosides are partly soluble in amyl alcohol, but can be removed from it by extraction with very dilute aqueous acid. Other methods of separation have been utilised, *e.g.* a method based upon the varying solubilities of the picrate oxonium salts in organic solvents, and chromatographic adsorption.

Various anthocyanidins may be distinguished by (i) *oxidation*—when shaken with air in dilute sodium hydroxide, petunidin and delphinidin colours are destroyed, the others being stable—and (ii) by the colour with *ferric chloride* in amyl alcohol solutions.

EXPT. 73. *Detection of Anthocyanins*

Extract any blue, red, or purple flower-heads with aqueous alcohol on a water-bath, decant the solution, and divide it into four portions. (*a*) Add acid: a red colour is produced. Then add alkali: the colour changes to blue or green. The latter colour is due to the blue of the anthocyanin being superimposed on the yellow of anthoxanthins also present. Acidify once more: the red colour returns. (*b*) Add ferric chloride solution: colorations varying from blue to violet are obtained, unless much anthoxanthin is present, in which case a green colour will be developed. (*c*) Add a little cold dilute sulphuric acid, then amyl alcohol equal in volume to the solution, and shake. The colour remains in the aqueous layer, showing that anthocyanin (glycoside) is present. (*d*) Add a little sulphuric acid, warm gently, then cool and shake with amyl alcohol. The anthocyanidin which has been formed on hydrolysis goes into the amyl alcohol layer.

Exceptional anthocyanin-like pigments, the structures of which have not all been completely elucidated, include the nitrogen-containing pigment **betanin** from Beetroot (*Beta vulgaris*) and a similar pigment in *Celosia cristata* and in Winter Spinach (*Atriplex hortensis atrosanguineus*). They appear to be hydroxy-derivatives of 4′-aminoflavylium (p. 211). Yellow anthocyanins have also been obtained from *Papaver alpinum*, from the Iceland Poppy (*Papaver nudicaule*), and from the Welsh Poppy (*Meconopsis cambrica*).

A spectrographic method has been introduced for identifying the anthocyanidin in plant extracts, but isolation and usually synthesis are necessary to determine the exact positions of the sugar residues in the anthocyanin molecule.

There follows an account of the distribution of the various anthocyanins in plants, grouped according to their derivation from the three anthocyanidin nuclei, and thereafter according to their glycosidic residues (p. 210).

Pelargonidin Derivatives. Pelargonidin itself gives no characteristic colour with ferric chloride, but gives a blue precipitate with lead acetate.

3-Glucoside: **Callistephin,** in flowers of the purple-red Aster (*Callistephus chinensis*) and of scarlet Carnations (*Dianthus Caryophyllus*). It was the first anthocyanin to be synthesised.

3-Galactoside: **Fragarin,** in the fruit of the Strawberry (*Fragaria*).

3-Rhamnoglucoside: in the scarlet *Gloxinia.*

Diglucoside: **Punicin,** in leaves of the Pomegranate (*Punica granatum*).

3, 5-Dimonoglucoside: **Pelargonin,** in flowers of scarlet Geranium (*Pelargonium zonale*) to 7 per cent. of the dry weight; also in the scarlet Dahlia (*Dahlia variabilis*), in the rose-pink variety of the Cornflower (*Centaurea Cyanus*), in the scarlet *Gladiolus,* and probably in other scarlet flowers. Partial hydrolysis yields the 5-glucoside, pelargonenin.

Complex derivatives: **Salvianin** in flowers of the scarlet Salvia (*Salvia splendens*) and **monardæin** from the Golden Balm (*Monarda didyma*) both of the *Labiatæ,* are identical, and on complete hydrolysis yield pelargonidin, two molecules of glucose, one molecule of *p*-hydroxycinnamic acid, and two molecules of malonic acid. The *p*-hydroxy-cinnamic acid is condensed with one of the phenol groups (probably in position 7), and two molecules of the monomethyl ester of malonic acid ($HOOC{\cdot}CH_2{\cdot}COOH_3$) are attached to the sugar residue.

Cyanidin Derivatives. Cyanidin in alcoholic solution gives a blue colour, in aqueous solution a violet colour, with ferric chloride. In alcoholic solution it also gives a blue precipitate with lead acetate.

3-Glucoside: **Chrysanthemin** in red varieties of *Chrysanthemum indicum* and **asterin** in the purple-red Aster (along with callistephin) are identical; also in fruit of Blackberry (*Rubus fruticosus*), and of the Elderberry (*Sambucus nigra*).

3-Galactoside: **Idæin** in the skins of the Cowberry (*Vaccinium Vitis-idœa*).

3-Rhamnoglucoside: **Antirrhinin,** from flowers of *Antirrhinum majus.* Partial hydrolysis gives rhamnose and chrysanthemin. **Keracyanin** in skins of the sweet Cherry (*Prunus avium*) is probably identical. **Prunicyanin** from fruit of the Sloe or Blackthorn (*Prunus spinosa*) also gives rhamnose and glucose on complete hydrolysis.

3-Gentiobioside: **Mecocyanin,** in flowers of purple-scarlet garden variety of the Poppy (*Papaver Rhœas*). Partial hydrolysis gives chrysanthemin and glucose.

3, 5-Diglucoside: **Cyanin,** in the blue Cornflower occurs to 0·75 per cent. of the dried flowers; in the deep purple variety of the Cornflower to 14 per cent., to 20 per cent. in the deep red Dahlia, and 2 per cent. in *Rosa gallica*; also in many other flowers.

3′-Monomethyl ether of Cyanidin, or **Peonidin derivatives:**

3-Glucoside: **Oxycoccicyanin** in Cranberries (*Vaccinium oxycoccos*).
3, 5-Diglucoside: **Peonin** in deep red flowers of Peony (*Pæonia officinalis*).

Delphinidin Derivatives. Delphinidin chloride is soluble in both methyl and ethyl alcohols, giving a purple solution. This changes to a stable blue colour on addition of ferric chloride solution, but the latter gives an unstable violet coloration with an aqueous solution of the pigment. Some of the derivatives of delphinidin, *e.g.* malvin, give no coloration in aqueous solution with ferric chloride. Lead acetate gives a blue precipitate with an alcoholic solution of delphinidin.

Monoglycoside: **Vicin** in dark-red flowers of Vetch (*Vicia*) is probably a mixture of a monorhamnoside and a monoglucoside.
3, 5-Diglucoside: in flowers of *Salvia patens.*
Complex Derivatives:

Gentianin, in the Alpine Gentian (*Gentiana acaulis*) gives on hydrolysis equimolecular amounts of delphinidin, *p*-hydroxy-cinnamic acid, and glucose.

Violanin, in the blue-black Pansy (*Viola tricolor*) to 24 per cent. of the dry weight of the flowers; also in Violas. On hydrolysis, it yields delphinidin, glucose, rhamnose, and *p*-hydroxy-cinnamic acid.

Delphinin, in flowers of Larkspur (*Delphinium consolida*) is a diglucoside and also gives two molecules of *p*-hydroxy-benzoic acid on hydrolysis.

Of the pigments which are **methyl ethers** of **delphinidin,** it is not yet known whether some are true diglycosides or mixtures—as others definitely are—of delphinidin monoglycosides and delphinidin methyl ether glycosides.

3′, 5′-Dimethyl ether of Delphinidin, or **Malvidin derivatives:**

3-Glucoside: **Œnin** (or **Primulin**) in black Grapes (from *Vitis vinifera*) in flowers of *Primula polyanthus*, in several varieties of *Primula sinensis*, and in some *Cyclamens.*
3, 5-Diglucoside: **Malvin** in violet flowers of the Mallow (*Malva sylvestris*), and in flowers of *Primula viscosa* and in *P. integrifolia.*
Mixtures: Ampelopsin from berries of the Virginian Creeper (*Ampelopsis quinquefolia*), and althæin from purple flowers of Hollyhock (*Althœa rosea*) are mixtures of monoglucosides of delphinidin and malvidin. Similarly myrtillin from fruit of the Whortleberry (*Vaccinium Myrtillus*) on hydrolysis gives

delphinidin, malvidin, glucose, and galactose. The pigment of American grapes (from *Vitis riparia*) gives on hydrolysis a methyl ether of delphinidin, glucose, and *p*-hydroxy-cinnamic acid.

7, 3′, 5′-Trimethyl ether of delphinidin, or **Hirsutidin derivatives:**

3, 5-Diglucoside: **Hirsutin** in *Primula hirsuta.*

Distribution, Function, and Relationship of the Soluble Pigments. The anthoxanthins are very widespread in leaves, flowers, and stems, especially in the epidermis of plants growing in exposed situations; their function in protecting the plant from over-insolation is therefore one explanation of their presence, both chlorophyll and the enzyme diastase being light-sensitive. It has been shown that among both alpine and tropical plants, those plants grown in the shade have less flavone than those grown in the open, and that increased insolation either in more sunny habitats or at higher altitudes leads to an increase in flavone content. A similar protective function has been advanced in explanation of the presence of anthocyanins in young shoots and buds. Another suggestion for the function of anthocyanins occurring in leaves is that by converting the absorbed rays into heat they actually raise the temperature of the tissue. The anthocyanins may also have the biological function of attracting insects to flowers for pollination, and birds to fruits for their distribution.

The distribution of the various cell-sap pigments in plants has been studied. Mixtures of related anthoxanthins in one plant are common, *e.g.* 'Persian berries' (*Rhamnus*) contain quercetin, together with its dimethyl ether rhamnazin, and its monomethyl ether glycoside, xanthorhamnin. In some cases different pigments are found in different tissues of the same plant, *e.g.* the leaves of 'young fustic' (*Rhus*) contain myricetin, while the stems and branches contain fustin. The flowers from different varieties of Cotton contain different but related flavonols: Indian Cotton (*Gossypium herbaceum*) and the yellow-flowered *Gossypium neglectum* contain gossypitrin and *iso*quercitrin; the yellow flowers of the Sea Island cotton, *G. barbadense*, contain quercimeritrin in addition; and flowers of the American *G. hirsutum* contain quercimeritrin and *iso*quercitrin. It will also be seen from the distribution of the anthocyanins in flowers that species of the same genus, and even varieties of the same species, differ not only in their content of anthocyanin—which modifies the colour, *e.g.* in Cornflowers—but also in the actual anthocyanins present. The different species of *Primula* contain different delphinidin derivatives, while cyanin and pelargonin predominate in different coloured varieties of *Pelargonium zonale*. Again, a Nasturtium was found to contain a pelargonidin derivative in the flower petals, a cyanidin derivative in the calyx, and a delphinidin derivative in the leaves. Robinson has shown that since their cell-sap is usually acid, flowers of red, blue, and violet hues do not owe their colour to the difference in p_H of the cell-sap. This at first seemed probable, because these colours can all be given by one pigment at different p_H values. The main factors affecting the colour are (*a*) the *state of aggregation* of the pigment, allowing blue complexes such as cyanin to form in the acid cell-sap of the Cornflower; (*b*) association of the pigment with other molecules or *co-pigments*, which include the anthoxanthins and tannins. The slight differences in p_H which exist will of course modify the colour, as may also the presence of metallic salts, iron and aluminium being used in horticultural practice in the 'bluing' of Hydrangeas.

It has already been noted that although the anthocyanidins may be derived in the laboratory from flavonols by reduction, in most cases related pairs of compounds do not occur in the same plant. It would therefore seem more likely that both groups of soluble pigments are built up in a similar way from the same original structure. Onslow has shown that anthocyanins appear when the content of carbohydrates is high. Robinson points out that all the soluble pigments contain the fundamental carbon skeleton C_6——C_3——C_6, probably derived from hexoses, the C_3 unit occurring by the cleavage of a hexose molecule and undergoing an aldol condensation with a hexose unit. This would give by subsequent oxidations and reductions not only the benzopyrone and benzopyrylium nuclei, but also such compounds as cinnamic acid, coumarin, coniferyl alcohol, and caffeic acid. Or, to put it somewhat differently, the γ-pyrone and pyrylium rings have the same fundamental structure as glucopyranose. In the pigments, a further condensation takes place with an aromatic nucleus, which is most commonly phloroglucinol, and which may also be derived from a hexose (p. 179). A common chromogen may therefore be developed for the pigments, and genetical relationships between flower colours may be attributed to factors which control the introduction of additional hydroxy- or methoxy-groups. The sugar residues, like those of most glycosides, are probably attached last.

CHAPTER XX

THE ALKALOIDS

Occurrence. The alkaloids have been used since early times for both medicinal and lethal purposes, but it was not until after the isolation of 'morphia' from opium in 1817 by Sertürner and the discovery of other similar *alkali-like* substances of plant origin that the term alkaloid became established. The alkaloids may be defined as relatively complex and physiologically active plant bases containing cyclic nitrogen. Many of them are extremely poisonous. They are restricted to a comparatively few natural orders in the dicotyledons, particularly the *Apocynaceæ*, *Leguminosæ*, *Papaveraceæ*, *Fumariaceæ*, *Ranunculaceæ*, *Rubiaceæ*, and *Solanaceæ*. The *Gramineæ*, *Labiatæ*, and *Rosaceæ* rarely contain alkaloids, while the *Compositæ* are intermediate. Alkaloids are almost entirely absent from the Cryptogams. Sometimes an individual alkaloid may be characteristic of its order, *e.g.* protopine is widely distributed in the *Papaveraceæ* and in the allied order *Fumariaceæ*; usually also the members of the same order contain closely related alkaloids. Often, however, the alkaloid is characteristic of one genus or even of one species only. Thus each botanically distinct species of *Aconitum* so far examined contains a distinct alkaloid, but these various *aconitines* are all closely related chemically. In young tissues, the alkaloids may be present in solution in the cell-sap, but they also accumulate in the solid form in mature tissues such as seeds, fruits, roots, and bark. In the few instances such as Hemlock, Poppy, and some of the *Solanaceæ*, which have been completely examined for alkaloids, these are found distributed throughout the whole plant; the amount varies in the different tissues, and in each tissue with the age of the plant, season, etc. Special cultivation and selection can increase the quantity of alkaloid elaborated by plants, as has been done for *Cinchona* bark.

Isolation and Properties. The alkaloids are generally colourless, crystalline substances, containing carbon, hydrogen, oxygen, and nitrogen; a few, such as coniine and nicotine, contain no oxygen, and these are generally volatile liquids. Only a few alkaloids are coloured, *e.g.* berberine, which is yellow. The alkaloids are generally insoluble in water, but soluble in the common organic solvents, *viz.* ether, chloroform, and alcohol. In a few rare cases, the alkaloid occurs in the free state in the plant, and therefore can be isolated by extraction with these solvents. Generally they occur as **salts with organic acids,** *e.g.* malic, citric, and succinic acids, and occasionally with special complex organic acids, *e.g.* quinine with quinic acid (p. 179). Most plants in which alkaloids occur contain a mixture of several chemically related alkaloids. Two general methods of extraction are used: (*a*) mixing the ground plant tissue with lime or magnesia and extracting the liberated alkaloid with an organic solvent, or, as with nicotine and coniine, volatilising it by steam distillation; (*b*) extracting the alkaloid as a salt with dilute mineral acid, and then precipitating the base with a weak alkali such as sodium carbonate or ammonia. In both cases the crude alkaloids can be purified through their salts with inorganic or organic acids, *e.g.* halides or picrates. The alkaloids also form double salts with many of the metallic halides, *e.g.* with auric, platinic, and mercuric chlorides. Other precipitating reagents are tannic acid, picric acid, phosphotungstic acid, etc., and these are called the *alkaloid reagents*, although any one alkaloid does not necessarily give all these reactions. In the alkaloids, the *basic properties* predominate, but they may contain other typical groups, and exhibit varied properties due to these, *e.g.* atropine is also an ester. Many of the alkaloids give distinctive colour reactions with concentrated acids. The majority of the alkaloids are *laevo*-rotatory, but a few have a *dextro*-rotation and some are optically inactive.

General Tests for Alkaloids

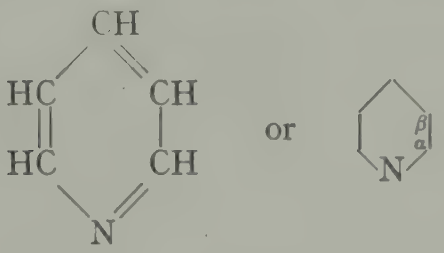
With solutions of the alkaloids or their salts—

(*a*) tannic acid solution gives a white precipitate,
(*b*) picric acid solution gives a yellow precipitate,
(*c*) iodine in potassium iodide solution gives a brown precipitate,
(*d*) mercuric iodide in potassium iodide solution gives a white precipitate,
(*e*) phosphotungstic acid solution gives a white precipitate.

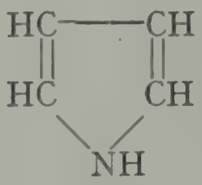
EXPT. 74. *Test for Alkaloids in Plant Tissue*

Pound the tissue in a mortar with 1 per cent. sulphuric acid, leave for some time to destroy any chlorophyll present, then filter. Use the filtrate for the above general tests. Suitable material includes leaves of Yew, pods of Broom, Laburnum, and Lupin, any part of Henbane or the Deadly Nightshade.

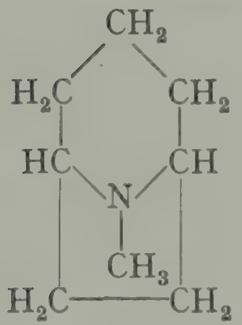
Structure. The alkaloids are usually classed according to the heterocyclic nitrogen-containing nuclei of which they are derivatives. Such an arrangement also groups most of the alkaloids according to the botanical classification of the plants from which they are derived. Since, however, some alkaloids from closely related plants differ chemically only in ring closure, they are classed in different groups and their chemical relationship is thereby obscured. Several of the betaïnes contain heterocyclic rings and are often classed with the alkaloids, as are also the purines. While caffeine, theobromine, etc., justify this inclusion, other purines are universally distributed in plants in the nucleic acids, and their basic character does not predominate, as in the alkaloids. The alkaloids may be grouped with reference to the following five heterocyclic nuclei:—



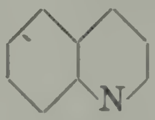
(I)
Pyridine



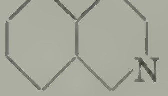
(II)
Pyrrole



(III)
Tropane



(IV)
Quinoline



(V)
*iso*Quinoline

Complete formulæ are known for the simpler alkaloids only; and these have been proved by synthesis. The structure of many of the more complex alkaloids still awaits elucidation. Much of what has been accomplished is due to Ladenburg, Skraup, Willstätter, Pictet, Perkin, and Robinson. Most plants in which alkaloids occur contain a mixture of such substances belonging to the same group; therefore in each group the alkaloids will be discussed according to the plants from which they are derived.

Group I. Pyridine Alkaloids

It will be seen from formula (I) that pyridine consists of an unsaturated six-membered ring similar to benzene, but with one —CH= group replaced by a nitrogen atom. This last is tervalent and can form salts with acids by becoming quinquevalent, as in the simple amines. Pyridine itself is obtained commercially in the distillation of coal tar. When pyridine is reduced, *i.e.* when all the double bonds in the molecule are saturated with hydrogen, the compound **piperidine,** $C_5H_{11}N$, is obtained. The alkaloids derived from pyridine and piperidine are as follows:—

(i) Alkaloids of Hemlock (*Conium maculatum*). The leaves and unripe fruits contain five alkaloids, the most important being **coniine,** a liquid obtained by mixing the crushed fruit with sodium carbonate solution and steam-distilling. Coniine is $C_8H_{17}N$, and is α-*n*-propyl-piperidine, $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}C_5H_{10}N$. It is the simplest alkaloid, and was the first to be synthesised. All the Hemlock alkaloids are poisonous. A similar mixture of alkaloids occurs in Fool's Parsley (*Æthusa Cynapium*).

(ii) Alkaloids of the root bark of the Pomegranate Tree (*Punica granatum*). The most important of this group of about five is **pelletierine,** $C_8H_{15}ON$. It is the propionaldehyde derivative of piperidine, $CHO{\cdot}CH_2{\cdot}CH_2{\cdot}C_5H_{10}N$, and is therefore the aldehyde corresponding to coniine.

(iii) The alkaloid of species of *Piper* is **piperine,** $C_{17}H_{19}O_3N$, which occurs to the extent of 5 to 9 per cent. in the fruit of *Piper nigrum* (used in the preparation of black and white pepper), and from 1 to 2 per cent. in Long Pepper (*Piper longum*). It is the amide (p. 140) of piperidine with a complex acid, piperic acid.

(iv) Alkaloids of tobacco, the dried leaves of the cultivated varieties of *Nicotiana tabacum*. The chief alkaloid is **nicotine,** $C_{10}H_{14}N_2$, which occurs in tobacco (0·6–10 per cent.), and is isolated as a *laevo*-rotatory liquid with a disagreeable odour. It is extensively used (*a*) in aqueous solution with soft soap as a spray, and (*b*) adsorbed on inert substances to give 'nicotine dusts', in the control of insect pests in orchards and gardens. Structurally, nicotine combines Groups I and II, as it is pyridine substituted in the β-position by *n*-methyl-pyrrolidine.

EXPT. 75. *Nicotine*

Show that nicotine is soluble in water giving an alkaline solution, and try the general tests (p. 219) on this aqueous solution.

(v) Alkaloids of the Areca Nut or Betel-nut Palm (*Areca catechu*), of which **arecoline,** $C_8H_{13}O_2N$, is the most important.

Other alkaloids of Group I are **ricinine,** $C_8H_8O_2N_2$, in the seeds and seedlings of the Castor Plant (*Ricinus communis*), and **trigonelline,** which is also a betaïne (p. 128); also the alkaloids from 'Indian tobacco' (*Lobelia inflata*), including **lobelanine, lobeline,** and others.

Group II. Pyrrole Alkaloids

Pyrrole, C_4H_4NH, formula (II), p. 220, and the corresponding saturated compound, pyrrolidine, C_4H_8NH, are the parent substances of a small group of alkaloids. Pyrrolidine itself occurs in small quantities in leaves of Carrot (*Daucus Carota*) and in tobacco, in the latter with *n*-methyl-pyrrolidine and nicotine. The most common alkaloids of this group are also betaines, *viz.* **stachydrine, betonicine,** and **turicine** (p. 128). Coca leaves (*Erythroxylon Coca*) contain important alkaloids of Group III, associated with small amounts of **hygrine,** $C_8H_{15}ON$, and **cuskhygrine,** which belong to Group II.

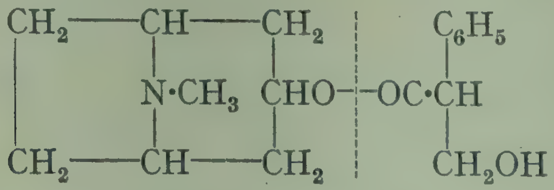
Group III. Tropane Alkaloids

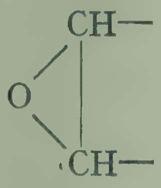
Tropane, formula (III), may be regarded as formed by the condensation of a piperidine and a pyrrolidine ring. The important members of this group occur in three natural orders.

(i) Alkaloids of the *Solanaceæ*. Most, but not all, of the alkaloids occurring in the *Solanaceæ* belong to the tropane group, and they are distinguished physiologically by their *mydriatic* effect (dilation of the pupil of the eye). The most important are **atropine, hyoscyamine,** and **hyoscine,** and these occur as a mixture in the following plants, the parts used for drugs being indicated in brackets, although often the alkaloids occur in all the tissues:—

Deadly Nightshade (*Atropa Belladonna*)—chiefly hyoscyamine (leaves and roots).
Thorn Apple (*Datura Stramonium*)—chiefly hyoscyamine (leaves).
Datura fastuosa—chiefly hyoscine (leaves and seeds).
Datura arborea and *Datura Metel*—chiefly hyoscine.
Datura meteloides—hyoscine, atropine, meteloidine, *nor*hyoscyamine.
Henbane (*Hyoscyamus niger*)—chiefly hyoscyamine, also hyoscine and atropine (leaves).
Hyoscyamus muticus and *H. reticulatus*—chiefly hyoscyamine.
Hyoscyamus albus—hyoscyamine and hyoscine.
Scopolia atropoides—hyoscyamine and hysocine.
Scopolia japonica—hyoscyamine and *nor*hyoscyamine.

All these alkaloids are esters (tropeïnes) of tropic, atropic, and tiglic acids with the basic alcohols, tropine, *nor*tropine, scopine, etc. Warming with dilute alkali or acid hydrolyses hyoscyamine and atropine, $C_{17}H_{23}O_3N$, to *tropine*, $C_8H_{15}ON$ (which is both a base and a secondary alcohol), and an acid, *tropic acid*, $C_9H_{10}O_3$; hyoscyamine yields *l*-tropic acid, while atropine yields an optically inactive tropic acid. Hence hyoscyamine and atropine are stereoisomeric esters represented by the formula:





Hyoscine, or scopolamine, $C_{17}H_{21}O_4N$, is similarly built up from tropic acid and scopine, an oxidation product of tropine, the alteration in the formula being shown above.

(ii) Alkaloids from the genus *Erythroxylon*.—Leaves of the Coca plant (*E. Coca*) contain a mixture of alkaloids related to each other and also to the Solanaceous alkaloids, the chief members being **cocaine** and **tropacocaine.** Most of them, like those of the previous group, are esters. Cocaine, $C_{17}H_{21}O_4N$, on hydrolysis yields benzoic acid, methyl alcohol, and ecgonine, which is tropine with a carboxyl group on a carbon atom adjacent to the alcoholic group. In cocaine itself, the alcoholic group is esterified with benzoic acid, and the carboxyl group is methylated.

The physiological effects of these tropeïnes differ: atropine and *l*-hyoscyamine are chiefly mydriatic; cocaine and tropacocaine produce local anæsthesia; while hyoscine is mainly used for its sedative effect.

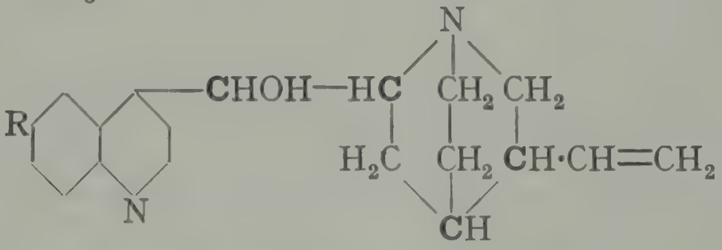
Hygrine (p. 222) also occurs in Coca leaves, and furnishes an example of compounds closely related chemically and occurring in the same plant, yet separated by this classification; another example is provided by one of the Pomegranate alkaloids, *pseudo***pelletierine,** which is a tropane alkaloid. In both cases the difference is one of ring closure.

(iii) Some Alkaloids of the *Papilionaceæ*.—This type contains a more complex nucleus based on tropane, or alternatively it may be regarded as a bridged piperidine ring (*vide infra*), and is sometimes called the **quinuclidine** sub-group. To it belong **sparteine,** $C_{15}H_{26}N_2$, from the flowering tops of Broom (*Cytisus scoparius*), which is identical with **lupinidine** from seeds of yellow Lupin (*Lupinus luteus*), also **genisteine,** $C_{16}H_{28}N_2$, from *Genista* and probably other, but not all, alkaloids from this sub-family.

Group IV. Quinoline Alkaloids

The alkaloids containing the quinoline nucleus, formula (IV) (p. 220), can be divided into two groups both chemically and also according to their botanical origin: (i) the **quinolyl-quinuclidine** type in the *Rubiaceæ*, and (ii) the alkaloids of species of *Strychnos*.

(i) The bark of species of the genera *Cinchona*, 'cinchona bark,' and *Remijia*, 'cuprea bark,' of the *Rubiaceæ*, all indigenous to South America, but now cultivated on a large scale in Java and India, yield alkaloids of this sub-group. More than twenty alkaloids have been isolated from cinchona bark, of which the most important are **quinine, quinidine, cinchonine,** and **cinchonidine,** occurring as salts with specific acids, *quinic acid* (p. 179) and *cinchotannic acid*. Cuprea bark contains quinine and **cupreine.** The molecules of these alkaloids contain two nuclei, the quinoline and the quinuclidine, and the general formula for the cinchona alkaloids is given below; it contains four asymmetric carbon atoms and therefore many isomers are possible. Cinchonine and cinchonidine are isomeric and R = H, while quinine and quinidine are isomeric, and R = OCH_3.



Quinine is a colourless crystalline solid, only slightly soluble in cold water. It is *laevo*-rotatory, and is valuable medicinally as a febrifuge. Its most characteristic salt is the sulphate, which dissolves in water to give a solution with a blue fluorescence.

Expt. 76. *Properties of Quinine*

(i) Try the general tests (p. 219) with an aqueous solution of quinine sulphate.

(ii) To a solution of the sulphate add some bromine water, and then ammonia; a green coloration or precipitate is formed.

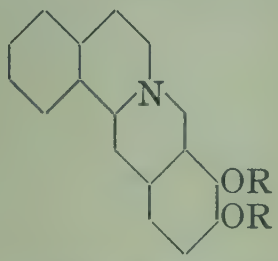
(iii) Place a solution of the sulphate in a separatory funnel, add dilute sodium hydroxide solution until alkaline, and shake. Quinine is precipitated. Then add chloroform, and show that the quinine dissolves and goes into the lower chloroform layer.

(ii) The *Strychnos* alkaloids: These include **strychnine,** $C_{21}H_{22}O_2N_2$, and **brucine,** $C_{23}H_{26}O_4N_2$, a mixture of which occurs to the extent of 2–3 per cent. in the seeds of Nux Vomica (*Strychnos Nux-vomica*), found in the East Indies, and in St. Ignatius' Beans (*S. Ignatii*) of the Philippine Islands; while **curine,** $C_{18}H_{19}O_3N$, and **curarine,** $C_{19}H_{26}ON_2$, occur in the arrow poison 'curare' from South American species of *Strychnos* (*e.g. S. toxifera*). Both strychnine and brucine are *laevo*-rotatory, and brucine is dimethoxy-strychnine. They contain a very complex ring structure which includes a quinoline nucleus. Strychnine is exceedingly poisonous, brucine less so, while curare is only poisonous when injected.

Group V. *iso*Quinoline Alkaloids

The *iso*quinoline alkaloids may be divided into two large groups, *viz.* (i) the berberine alkaloids, and (ii) the opium alkaloids. They all contain a potential *iso*quinoline nucleus, but sometimes in a much larger ring system. The berberine alkaloids are derivatives of di-*iso*quinoline, whereas the opium alkaloids can be divided into the papaverine sub-group, which contains the *iso*quinoline nucleus, and the morphine sub-group, which contains a potential *iso*quinoline group in a condensed ring system.

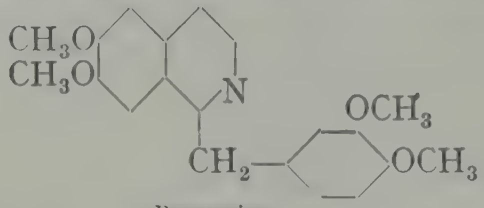
(i) The Berberine or Di-*iso*quinoline Alkaloids:



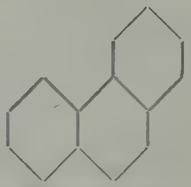
The root barks of the common Barberry (*Berberis vulgaris*) and of the American species, *B. aquifolium*, contain a mixture of three alkaloids, namely, **berberine,** $C_{20}H_{19}O_5N$, **berbamine,** $C_{18}H_{19}O_3N$, and **oxyacanthine,** $C_{19}H_{21}O_3N$. Berberine is widely distributed in plants; it occurs in the rhizomes of the Golden Seal (*Hydrastis canadensis*) and in *Coptis*, both of the *Ranunculaceæ*; in the roots and latex of Celandine (*Chelidonium majus*) of the *Papaveraceæ*; and in tropical species of the *Rutaceæ* and *Menispermaceæ*. The Celandine, the Yellow-horned Poppy (*Glaucium flavium*), and *Sanguinaria canadensis*, each contain mixtures of alkaloids, some of which are identical; all of them are related structurally to berberine. **Lycorine,** $C_{16}H_{17}O_4N$, another di-*iso*quinoline alkaloid, was first obtained from the Japanese plant, *Lycoris*; it occurs also in bulbs of various species of the family *Amaryllidaceæ*, including the wild Daffodil (*Narcissus Pseudo-narcissus*). Others are **corydaline** from *Corydalis cava* and *C. tuberosa*, and **hydrastine,** which occurs with berberine in the Golden Seal.

(ii) Opium Alkaloids: The dried latex obtained by making incisions in the unripe fruits of the Opium Poppy (*Papaver somniferum*) is known as *opium*, and its aqueous-alcoholic extract is called *laudanum*. Opium contains a mixture of some twenty-five alkaloids which can be divided into two sub-groups depending on their molecular structure.

(*a*) The papaverine sub-group contains **papaverine,** $C_{20}H_{21}O_4N$, and **narcotine,** $C_{22}H_{23}O_7N$, as the chief members. Oxidation and fusion with alkali in both cases splits the molecule into a basic *iso*-quinoline part and an acidic part. The formula for papaverine, which has been confirmed by synthesis, is shown below:



Papaverine

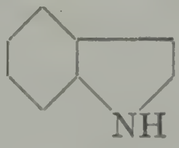


Phenanthrene

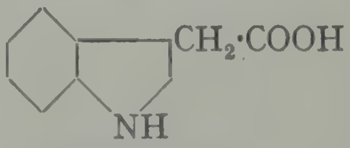
(*b*) The morphine sub-group contains **morphine,** $C_{17}H_{19}O_3N$, and **codeïne,** $C_{18}H_{21}O_3N$, as its chief members, codeïne being the methyl ether of morphine. The members of this sub-group are not themselves *iso*quinoline derivatives, but are modifications in which the nitrogen atom occurs in a complex ring system, sometimes containing also a phenanthrene nucleus; such compounds readily give *iso*quinoline derivatives, however, owing to the rupturing of some of the rings. Similar alkaloids are **colchicine** in the seeds and corms of the Autumn Crocus (*Colchicum autumnale*) and of related species.

Other Alkaloids

Several alkaloids are known which appear to have molecular formulæ derived from other nuclei than the five already discussed. For instance, the seeds of *Peganum harmala* contain the important alkaloids **harmaline,** $C_{13}H_{14}ON_2$, and **harmine,** $C_{13}H_{12}ON_2$, which are derivatives of *indole,* and are probably built up from the amino-acid tryptophan (p. 131). Indole rings can, however, arise in the plant by the oxidation of tyrosine by the enzyme tyrosinase (p. 261). **Indole** itself is one of the products of the putrefaction of proteins, but it also occurs in several volatile oils, *e.g.* from Orange and Jasmine flowers; the next higher homologue, methyl indole or



Indole



Indole-β-acetic acid

scatole, occurs in the wood of *Celtis reticulosa.* The hydroxy-derivative of indole, indoxyl, occurs in the glucoside indican (p. 112), and indole-β-acetic acid is one of the plant hormones (p. 306).

The *Solanum* alkaloids are unique in that they occur as glycosides, and the aglucone has a steroid nucleus (p. 55). **Solanine,** $C_{45}H_{73}O_{15}N$, from the Potato (*Solanum tuberosum*), on hydrolysis furnishes the trisaccharide rhamnosido-galactosido-glucose (which has not been found in any other natural product) and solanidine, $C_{27}H_{43}ON$. This has been partially synthesised from sarsasapogenin (p. 109). The alkaloids of *Veratrum* species are related to the *Solanum* group as they give some of the same degradation products.

A small group of alkaloids isolated from *Delphinium* and *Aconitum* have a diterpenoid structure (p. 243).

Physiological Function and Mode of Formation in Plants. Three possible functions have been allocated to the plant alkaloids by various investigators. (i) The alkaloids are forms of organic nitrogen used by the plant in various metabolic processes. Nicotine (but not all the other alkaloids of tobacco) is formed exclusively in the roots, and is translocated to the leaves, where it accumulates especially at the tips and edges, where translocation is at a maximum. This was shown by grafting Tobacco scions on Tomato roots, and *vice versâ.* This would indicate that nicotine is definitely not an end-product of nitrogen metabolism, but is an active metabolite. It may be connected with a supply of nicotinic acid to enzyme systems (p. 170). (ii) The alkaloids serve as protective materials in the plant against depradations by animals. (iii) The alkaloids are end-products of nitrogen metabolism, stored in a form relatively harmless to the plant, often in tissues which are discarded, *e.g.* seeds and fruit. This last idea is supported by an investigation of the Opium Poppy, in which the alkaloids are shown definitely to be waste products. Various theories as to the mode of formation of the alkaloids have been propounded. The simpler nitrogenous substances related to the alkaloids, *viz.* the amines and betaïnes, result from the decomposition of proteins; and this, the most generally accepted view for the source of the alkaloids as well, would account for their elaboration as by-products. Robinson has given a comprehensive scheme whereby the complex cyclic structures of the alkaloids can be derived from the *amino-acids*—especially lysine and ornithine (p. 136)—and decomposition products of the *carbohydrates*, by reactions which are known to occur in the plant, such as reduction, oxidation, dehydration, methylation, methylenation (that is, condensation with formaldehyde), aldol condensation (p. 58), and the similar condensation of hydroxy-amines, $=C(OH)\cdot N=$, with the grouping $=CH\cdot CO-$, by elimination of a molecule of water. Several syntheses of alkaloids from simple compounds by such methods have been achieved. For instance, the structure of all the *iso*quinoline alkaloids can be traced from the interaction of amino-benzaldehyde and its homologues with acetone and formaldehyde. Pictet has shown that a six-membered ring containing nitrogen can result by a molecular rearrangement from a methylated five-membered ring, *e.g.* pyridine from 1-methyl pyrrole, and quinoline from 1-methyl indole, but to what extent this reaction can or does take place in plants is not known.

CHAPTER XXI

THE ESSENTIAL OILS

Distribution

Almost all plants contain fragrant volatile products which can be isolated by steam distillation. These are termed the *essential oils*, in contradistinction to the fixed oils (p. 34). Closely allied chemically to these oils are (*a*) certain crystalline solids, such as camphor, menthol, and borneol, also volatile in steam; and (*b*) some non-crystalline solids usually obtained as exudations from plants and classed as balsams, resins, and rubber. The essential oils occur in various parts of the plant, sometimes in all organs, and sometimes in certain tissues only. They are found especially in flowers, *e.g.* Lavender; in fruits, *e.g. Citrus*; and in leaves, *e.g. Eucalyptus*; also to a less extent in bark, *e.g.* Cinnamon; and in wood, *e.g.* Sandalwood and Camphor wood. Essential oils occur in relatively large amounts in species of the *Coniferæ*, *Rutaceæ* (especially the genus *Citrus*), *Labiatæ*, *Myrtaceæ* (especially the genus *Eucalyptus*), and in the genus *Cymbopogon* of the *Gramineæ*. Many of these oils are of commercial importance. They are usually isolated by steam distillation, or by extraction with volatile solvents, or with fixed solvents (*enfleurage*), such as lard and vaseline. The essential oils are usually mixtures of compounds, often belonging to widely separated chemical classes, although in some instances one chemical individual may constitute more than 90 per cent. of the oil. Some of these oils contain *aromatic* compounds; but many of the constituents belong to a new class of compounds, the **alicyclic group.** Besides utilising the physical process of fractional distillation, separation of the chemical constituents of an oil includes the use of special chemical reagents such as sodium bisulphite for aldehydes and ketones, and calcium chloride for alcohols.

Before discussing the alicyclic compounds, a short list is given of some of the more important essential oils. It will be seen that the herbs once so common in English gardens figure in the list, as also do the spices of the Orient, while the incense woods owe their value to their content of balsams and resins (p. 242).

Coniferæ. 'Oil of turpentine,' from the sapwood of species of *Pinus*, *Abies* and *Larix*, contains mainly α- and -pinenes, and some *l*-limonene, and dipentene.

'Pine-needle oil,' from leaves of the same species, contains less pinene, and more *l*-limonene, dipentene, and bornyl acetate. 'Cedarwood oil,' from species of *Juniperus*, contains sesquiterpenes, especially cedrene.

Leaves of certain species of *Callitris*, native to Australia, contain geranyl acetate.

Rutaceæ. 'Lemon oil' from the peel of *Citrus Limonum* contains 90 per cent. of *d*-limonene and 4 to 5 per cent. of citral (the latter giving the characteristic odour).

'Orange oil,' from the peel of the Sweet Orange (*Citrus Aurantium*) and the Bitter Orange (*C. vulgaris*), contains *d*-limonene to 90 per cent.

'Neroli oil,' from Orange flowers, consists mainly of *d*-linalool and its acetate.

'Petitgrain oil,' from Orange leaves and shoots, also contains *d*-linalool and its acetate.

'Oil of Bergamot,' from the peel of *Citrus Bergamia*, contains *l*-linalool and its acetate and *d*-limonene.

Myrtaceæ. 'Clove oil,' from the flower-buds of *Eugenia caryophyllata*, cultivated in Zanzibar and Pemba, consists principally of eugenol (p. 177) and caryophyllene.

The genus *Eucalyptus* forms the major portion of the forest vegetation of the Australian continent, and more than 300 species have been distinguished botanically and chemically. They have been divided by Baker and Smith into groups depending on the chemical constituents of their leaves and stems, the following being representative types: *E. corymbosa* oil contains mostly pinene; *E. Australiana*, *E. globulus*, and *E. Smithii* oils contain more than 55 per cent. cineole, the remainder being largely pinene; *E. piperita* oil contains less than 40 per cent. cineole, with *l*-α-phellandrene and *l*-piperitone; *E. dives* oil contains about 50 per cent. *l*-piperitone, together with *l*-α-phellandrene; while *E. citriodora* oil contains 90 per cent. citronellal, and *E. Macarthuri* has 70 per cent. of geraniol mainly as acetate.

Other Australian genera of the *Myrtacæ* yielding oils of commercial importance are *Backhousia*, *e.g. B. citriodora* oil contains 94 to 97 per cent. citral, while *B. sciadophora* oil yields 80 to 85 per cent. α-pinene; *Leptospermum*; and *Melaleuca*, *e.g.* 'oil of cajuput' from both Australian and Indian species contains about 55 per cent. cineole.

Labiatæ. 'Oil of lavender' from flowers of *Lavandula vera* contains linalool and its acetate. 'Spike lavender oil' contains less linalool and more cineole, borneol, and some camphor. It is obtained from *L. spica*. Many hybrids of these species exist with corresponding differences in chemical content.

'Peppermint oil' from the stems and leaves of the Japanese *Mentha arvensis*, and the European *Mentha piperita* (also cultivated in the U.S.A.) consists of *l*-menthol (80 per cent.) and *l*-menthone. 'Oil of spearmint' from the American *Mentha viridis* contains *l*-carvone, while 'oil of pennyroyal' from *Mentha pulegium* grown in Europe yields *d*-pulegone.

'Rosemary oil' from flowers and leaves of *Rosmarinus officinalis* contains camphor and borneol.

'Oil of thyme,' from *Thymus vulgaris* and related species, yields thymol and carvacrol (p. 175) in varying amounts, and some species contain large amounts of citral in addition.

'Oil of sweet majoram' from *Origanum Majorana* contains a terpinenol, and various species of *Origanum* contain thymol and carvacrol as well.

'Oil of sage' from *Salvia officinalis* contains pinenes, cineole, and thujone.

'Oil of sweet basil' from *Ocimum basilicum* contains linalool and ocimene. Some other species of *Ocimum* contain thymol.

Umbelliferæ. 'Oil of caraway' from the carpels of *Carum Carvi* grown in Northern and Central Europe consists almost entirely of *d*-carvone.

'Oil of dill' from the fruit of *Anethum graveolens* contains *d*-carvone and *d*-limonene.

'Oil of coriander' from the fruit of *Coriandrum sativum* contains mainly linalool.

'Fennel oil' from the fruit of *Fœniculum officinale* contains from 80 to 90 per cent. anethole (p. 175).

'Oil of anise' from *Pimpinella anisum* also contains about 90 per cent. of anethole.

'Oil of celery' from all parts of the Celery plant, *Apium graveolens*, contains *d*-limonene (60 per cent.) and selinene.

Lauraceæ. The wood, twigs, roots and leaves of the Camphor tree (*Cinnamomum Camphora*), whose chief habitat is Formosa, contain *d*-camphor, which is isolated by sublimation or by steam distillation.

'Oil of cinnamom' from the bark of *Cinnamomum zeylanicum* (Ceylon) contains cinnamic aldehyde (75 per cent.) and a little eugenol, whereas the oil from the leaves consists mainly of eugenol.

'Oil of cassia' from the bark of *Cinnamomum cassia* (China) also consists of cinnamic aldehyde (90 per cent.) The difference between cinnamon and cassia oils (which accounts for the much higher value of the former) lies mainly in small traces of odorous constituents other than the aldehyde.

Gramineæ. The 'grass oils' from India and Ceylon come from species of *Cymbopogon* and *Andropogon*. 'Palmarosa oil' or 'Turkish geranium oil' from *Cymbopogon Martinii* contains geraniol; 'citronella oil' from *C. Nardus* contains citronellal; the oil from the Sudan 'Mahareb grass,' *C. sennaarensis*, contains 45 per cent. piperitone; while 'lemon-grass oil' or

'Malabar grass oil' from *C. flexuosus* contains 80 per cent. citral. The oil from *Andropogon Jwarancusa* contains *d*-piperitone.

Rosaceæ. 'Oil of roses,' 'otto,' or 'attar' of roses from the petals of *Rosa damascena*, cultivated in Bulgaria and France, contains geraniol and citronellol, with smaller quantities of many other constituents.

Geraniaceæ. 'Geranium oil' from the leaves of species of *Pelargonium* contains geraniol and citronellol and their esters.

Iridaceæ. 'Oil of orris root' is prepared from the rhizomes of three species of Iris, *I. pallida*, *I. florentina*, and *I. germanica*, and contains irone.

Ericaceæ. True 'oil of wintergreen' from the leaves of *Gaultheria procumbens* (p. 181) and commercial 'oil of wintergreen' usually from the bark of the Sweet Birch (*Betula lenta*) of the **Betulaceæ** consist principally of methyl salicylate.

TERPENES AND CAMPHORS

Alicyclic Compounds. When the unsaturated linkages in the benzene molecule are completely hydrogenated, the saturated compound *cyclo*hexane, C_6H_{12}, is obtained. The properties of *cyclo*hexane and its derivatives differ markedly from those of the corresponding unsaturated aromatic compounds, and in fact they resemble the paraffins in many reactions. Hence derivatives of *cyclo*hexane, and corresponding saturated ring systems containing from three to nine or more carbon atoms, are termed **alicyclic** compounds—that is, cyclic compounds resembling aliphatic substances. The **terpenes** and **camphors** are derived from *p*-methyl-*iso*propyl-*cyclo*hexane, $C_{10}H_{20}$, known as **menthane.** Hydrocarbons, alcohols, aldehydes, and ketones derived from menthane are all found in the essential oils. The term *terpene* is strictly applicable only to hydrocarbons, while the name *camphor* is used for the oxygenated derivatives. Many of the simple terpenes contain two unsaturated linkages, and for these the molecular formula is $C_{10}H_{16}$. The *sesquiterpenes* are a more complex group of compounds with the formula $C_{15}H_{24}$, while indiarubber belongs to the *polyterpenes*, $(C_5H_8)_n$. There are also present in essential oils certain unsaturated aliphatic compounds which only differ from the terpenes proper in lacking a closed ring; they are therefore often termed the *olefinic* (or *acyclic*) *terpenes*. Many of the terpene molecules contain one or more asymmetric carbon atoms; of the possible stereoisomers, sometimes only one form occurs; sometimes both active forms occur in different plants; and occasionally the inactive mixture also occurs. The chemistry of the terpenes owes much to the early researches of Wallach, Baeyer, Semmler, and W. H. Perkin, jun., and our knowledge of the structure of the sesquiterpenes to Ruzicka.

EXPT. 77. *Extraction of Essential Oils*

Place flowering heads of Lavender, or Eucalyptus leaves, or cloves, with a little water in a large round-bottomed flask set for steam distillation (fig. 6), and pass steam through the apparatus for one to two hours, when 'oil' will be found to separate in the receiver.

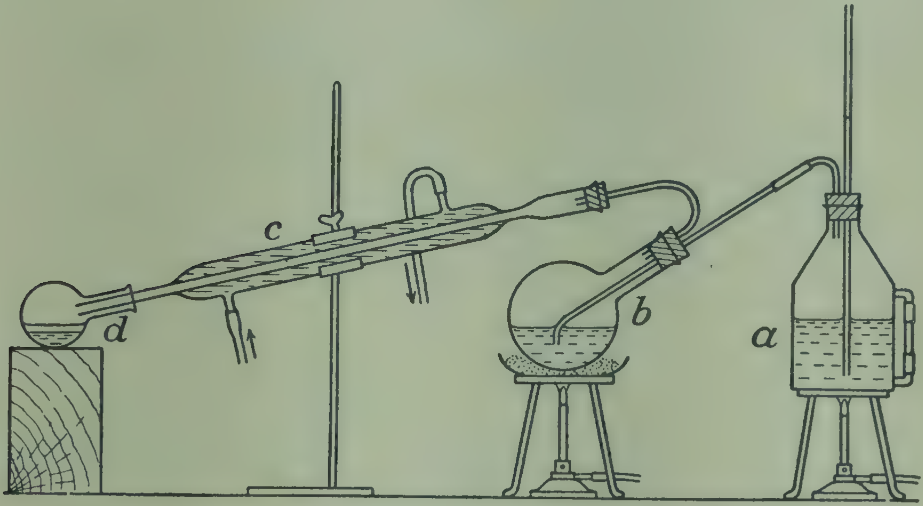
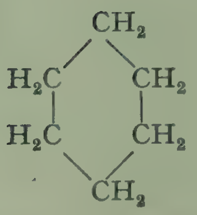


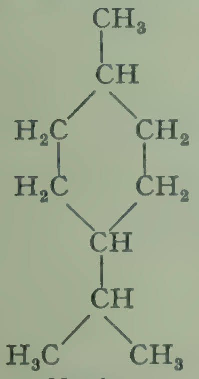
FIG. 6. Steam Distillation Apparatus.
(*a*) Steam can; (*b*) distillation flask, heated on a sand-bath; (*c*) condenser; (*d*) receiver

Menthol or 'mint camphor,' $C_{10}H_{20}O$, is a *secondary alcohol* derived structurally from menthane. The natural form occurring in 'peppermint oil' is *laevo*-rotatory. It is also prepared synthetically, mostly in the *dl*-form, by the reduction of piperitone and thymol. *l*-Menthol is a crystalline solid, m.p. 41°–43° C., with a peppermint odour, and is used as an antiseptic and mild anæsthetic.

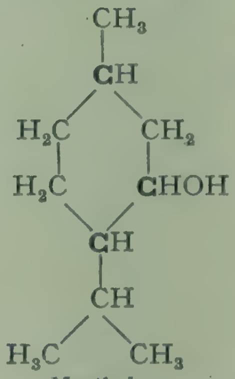
***l*-Menthone,** $C_{10}H_{18}O$, the corresponding *ketone,* occurs in the mother-liquors from the isolation of menthol by freezing, and is a liquid with a peppermint odour.



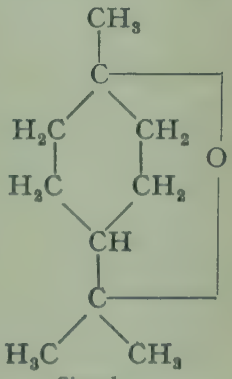
*cyclo*Hexane



Menthane



Menthol

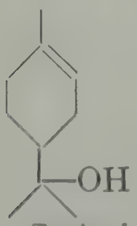


Cineole

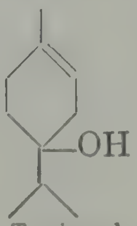
Cineole, or eucalyptole, $C_{10}H_{18}O$, is very widely distributed in essential oils, *e.g.* from species of *Eucalyptus* and *Melaleuca.* It has a characteristic 'eucalyptus' odour, and is used as a mild antiseptic. Structurally it is an *ether.*

Expt. 78. *Reactions of Cineole*

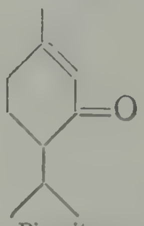
Place a few drops of cineole or of ordinary 'eucalyptus oil' in two test-tubes. To the first add a little syrupy phosphoric acid, when crystals of 'cineole phosphate' separate. Into the other pass bromine vapour and a solid bromo-derivative will be produced.



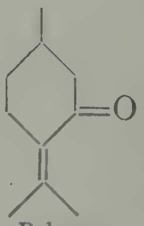
α-Terpineol



Terpinenol



Piperitone



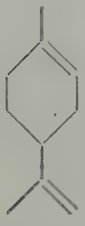
Pulegone

α-Terpineol, $C_{10}H_{18}O$, contains a tertiary *alcoholic* group and one unsaturated linkage. It occurs in the *d*-form in 'neroli' and 'petitgrain' oils, in the *l*-form in the oil from the Camphor tree, and in the *dl*-form in 'cajaput oil.'

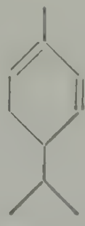
Terpinenol, $C_{10}H_{18}O$, which has the *alcoholic* hydroxyl group on a different carbon atom, occurs in 'oil of marjoram.'

Piperitone, $C_{10}H_{16}O$, an oil with a peppermint odour, is an unsaturated *ketone.* The *laevo*-form occurs to the extent of 50 per cent. in some *Eucalyptus* oils (especially *E. dives*), while the *dextro*-form occurs in species of *Andropogon* and *Cymbopogon*, and in Japanese 'peppermint oil.' It may be isolated by fractional distillation, or as the bisulphite compound (p. 58).

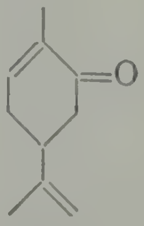
Pulegone, $C_{10}H_{16}O$, is a *ketone* isomeric with piperitone, differing in the position of the double bond. The dextro-form is widely distributed in the essential oils of the *Labiatæ*; the usual source is 'oil of pennyroyal' (p. 231).



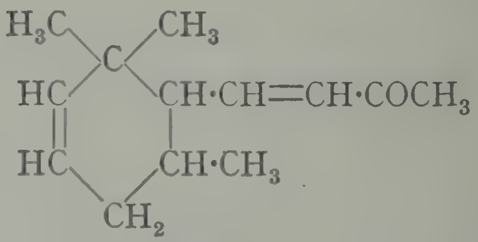
Limonene
Dipentene



α-Phellandrene



Carvone



Irone

Various natural terpene hydrocarbons are known containing two unsaturated linkages.

Limonene, $C_{10}H_{16}$, occurs in the *laevo*-form in some *Eucalyptus* oils, in 'pine-needle oil,' and in Russian 'turpentine oil,' while the *dextro*-form occurs in the oils of the *Rutaceæ*, and in oils of Celery and Dill. The optically inactive form, known as **dipentene** (= *dl*-limonene), occurs in some 'turpentine oils,' and is obtained by the destructive distillation of rubber.

α-**Phellandrene,** $C_{10}H_{16}$, is isomeric with limonene; the *l*-form occurs in some *Eucalyptus* oils, *e.g. E. phellandra* and *E. dives*, while the *d*-form occurs in 'fennel oil.'

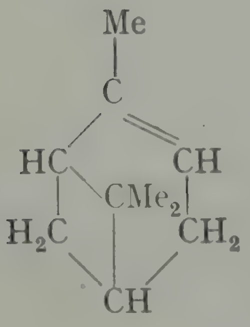
Carvone, $C_{10}H_{14}O$, is a *ketone* containing two other unsaturated linkages. It is widely distributed in essential oils, being the main component of 'caraway' and 'dill' oils. It is structurally related to carvacrol (p. 175), an aromatic alcohol, which often occurs with it in plants.

Irone, $C_{13}H_{20}O$, is a *cyclo*hexane derivative containing a *ketonic* group, and is the main constituent of 'oil of orris root.' **Ionone,** a synthetic substitute, differs from it in the position of the cyclic double bond.

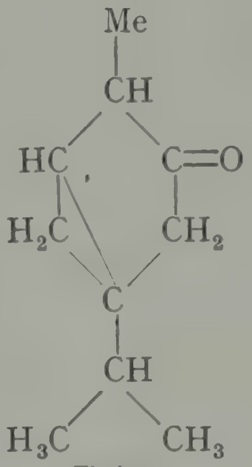
Alicyclic compounds are known containing *bridged cyclo*hexane rings. In some cases the *iso*propyl group is carried across the *cyclo*hexane ring and closed with one of the cyclic carbon atoms to give a *meta*- or a *para*-bridged ring.

α-**Pinene,** $C_{10}H_{16}$, occurs in most essential oils from the *Coniferæ*, and in particular it is the chief component of 'oil of turpentine.' It also occurs in many other plant oils. Turpentine is obtained by making incisions in the bark of species of *Pinus*, especially *Pinus palustris*, and is purified by steam distillation. The residue is rosin (p. 242). Both optically active forms and the racemic mixture of α-pinene occur naturally, often in different species of the same genus. *d*-α-Pinene occurs in American, Burmese, and Russian 'turpentine oils,' in oils of some species of *Eucalyptus*, in *Backhousia sciadophora*, and in oils of some of the *Umbelliferæ* (*e.g.* 'fennel,' 'carrot,' and 'coriander' oils); while the *l*-form is present in French 'turpentine oil' and 'lavender oil.' α-Pinene has the *meta*-bridged ring structure shown (p. 236 ; (Me = CH_3); β-pinene, which is isomeric with α-pinene, has the double bond outside the ring, forming a $C{=}CH_2$ group at the top carbon atom of the ring. *l*-β-Pinene occurs with α-pinene in many essential oils. Pinene is a good solvent; also it absorbs oxygen and resinifies, hence 'oil of turpentine' has a wide application in the preparation of varnishes and oil paints.

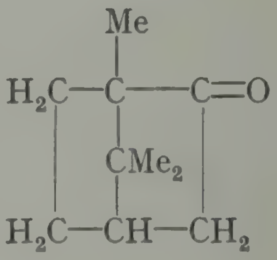
Thujone, $C_{10}H_{16}O$, is fairly widely distributed in essential oils, *e.g.* in 'tansy oil' (*Tanacetum vulgare*), and 'wormwood' or 'absinth' oil (*Artemisia Absinthium*), both of the *Compositæ*; in 'sage oil', and in 'thuja oil' (from *Thuja occidentalis* of the *Coniferæ*). It is a *ketone* containing an *iso*propyl group external to the *cyclo*hexane ring, and a simple *meta*-linking across the ring. It gives a crystalline bisulphite compound.



α-Pinene



Thujone



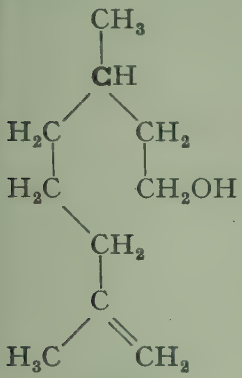
Camphor

Camphor, $C_{10}H_{16}O$, is another bicyclic *ketone*, the *iso*propyl group forming a *para*-bridged ring. The formula is usually written in the square form shown above. Camphor consists of colourless crystals with a characteristic odour, almost insoluble in water, and readily volatile. It has been used (in the East) since very early times for its medicinal properties. The *d*-form occurs in the Camphor tree, especially in the wood, while the *l*-form occurs in the leaf oil of *Blumea balsamifera*, and *dl*-camphor in *Chrysanthemum sinense.*

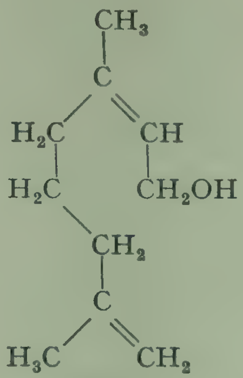
Borneol, or Borneo camphor, $C_{10}H_{18}O$, is the secondary *alcohol* corresponding to camphor. It is often present in the plant as esters, especially as bornyl acetate. The *dextro*-form occurs in the tree *Dryobalanops aromatica* of the *Dipterocarpaceæ*, often in such high concentrations that crystals are found in the wood, while the *laevo*-form occurs in *Blumea balsamifera* and in various other essential oils.

OLEFINIC TERPENES AND CAMPHORS

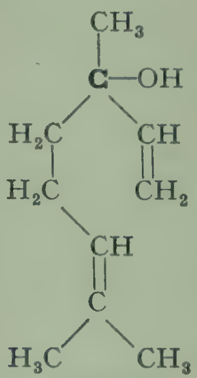
In addition to the true terpenes and camphors, many essential oils contain substances which are aliphatic unsaturated compounds structurally related to the former type, and known as the olefinic terpenes and camphors. By mere ring closure, such olefinic compounds may pass into alicyclic substances identical with or similar to those already discussed. To show this relationship, the formulæ of these aliphatic compounds are given below in the potential cyclic form:—



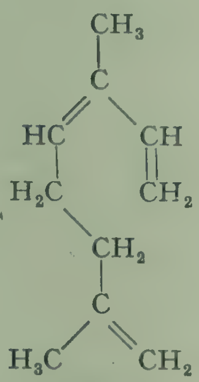
Citronellol



Geraniol



Linalool



Ocimene

Citronellol or rhodinol, $C_{10}H_{20}O$, is a primary *alcohol* containing one unsaturated linkage; it is probably in all cases a mixture of two isomers, the double bond being as shown above in one form, and between the adjacent carbon atoms in the other (as in the formula for linalool). The *laevo*-rotatory form occurs in 'rose oil' and 'geranium oil,' while the *dextro*-form is present in 'citronella oil.'

Citronellal, $C_{10}H_{18}O$, the corresponding *aldehyde,* occurs naturally in both the *dextro-* and *laevo*-form; it is found in oils from species of *Cymbopogon* and from *Eucalyptus citriodora.*

Geraniol, $C_{10}H_{18}O$, is a primary *alcohol* containing two unsaturated linkages. It is probably a mixture of two isomers with one double bond in different positions, as in citronellol. It occurs in 'rose oil,' and in 'Turkish geranium oil' from *Cymbopogon Martinii,* and is present both in the free state and as **geranyl acetate** in a large number of other essential oils, including *Eucalyptus Macarthuri* and *Darwinia fascicularis.* Geraniol and citronellol are the main components of 'attar of roses,' and their occurrence and isolation from other sources enable them to be used in many synthetic perfumes.

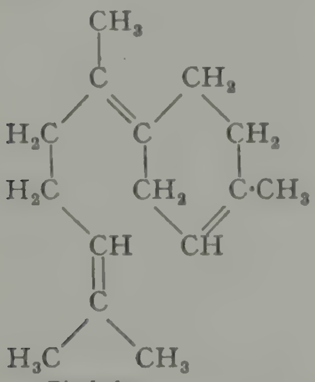
Citral, or geranial, $C_{10}H_{16}O$, is the *aldehyde* corresponding to geraniol. It occurs in the leaf oils of *Backhousia citriodora, Leptospermum Liversidgei, Eucalyptus Staigeriana,* etc.; it is the main constituent of 'lemon-grass oil' and also occurs in several of the *Citrus* oils.

Linalool, $C_{10}H_{18}O$, is a tertiary *alcohol* with two unsaturated linkages, and again there is a possibility of two positions for the lower double bond. Linalool, and its esters, especially the acetate, are present in 'oil of linaloe' from the Mexican tree *Ocotea candata* of the *Burseraceæ,* the *d*-form of linalool occurring in the seeds, and the *l*-form in the wood. The *d*-form also occurs in 'neroli' and 'petitgrain' oils and 'oil of coriander,' while the *l*-form occurs in 'bergamot' and 'lavender' oils.

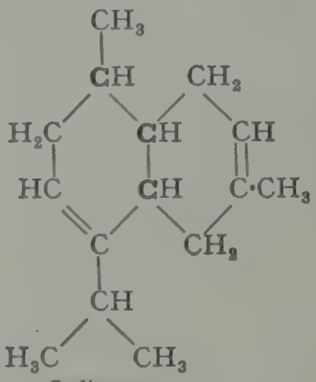
Ocimene, $C_{10}H_{16}$, is a *hydrocarbon* with three unsaturated linkages; it occurs particularly in species of *Ocimum, e.g.* 'oil of basil.'

SESQUITERPENES

The sesquiterpenes are *unsaturated alicyclic hydrocarbons* with the formula $C_{15}H_{24}$, some containing more than one ring system. Corresponding alcohols and other oxygenated derivatives also occur. The structure of the sesquiterpenes has only been elucidated in recent years, and in some cases the exact structural formula is not yet known.



Bisabolene



Cadinene

Bisabolene, $C_{15}H_{24}$, is one of the simplest sesquiterpenes, and its structure has been proved by synthesis. It is present in various essential oils, including 'bergamot oil.'

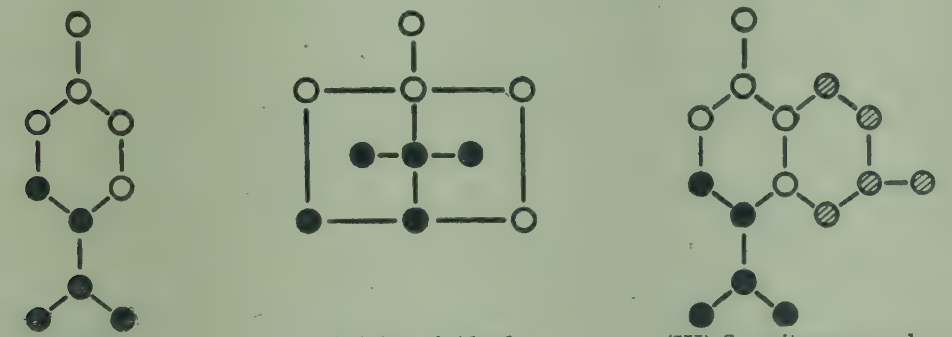
Other widely distributed sesquiterpenes are **cadinene,** in 'oil of cubebs' (from *Piper Cubeba*), and the **caryophyllenes** in 'oil of cloves.' Cadinene has a bicyclic structure, which is either as shown above, or with the double bond in the right-hand ring in the same position as in bisabolene. There are at least two caryophyllenes, but the complete structural formulæ have not yet been determined. **Selinene** from 'celery oil' is another bicyclic sesquiterpene; while **cedrene,** the main constituent of 'cedarwood oil,' has an unknown complex structure.

Santalol, $C_{15}H_{24}O$, the principal constituent of East Indian 'sandalwood oil' (from *Santalum album* of the *Santalaceæ*), and an important article of commerce for centuries, occurs in two forms, α- and β-, both primary *alcohols.* They are sesquiterpenes containing bridged rings.

POLYTERPENES AND ISOPRENE

Rubber is the coagulated latex obtained by making incisions in the trunks of several tropical trees and shrubs, especially of the genus *Hevea*. Rubber is a colloidal *hydrocarbon* of high molecular weight, with the formula $(C_5H_8)_n$. Dry distillation of rubber yields an *olefinic hydrocarbon*, **isoprene,** C_5H_8, with the branched-chain aliphatic structure, $CH_2{=}C(CH_3){\cdot}CH{=}CH_2$, which has been proved by synthesis. Isoprene undergoes polymerisation readily in the presence of catalysts such as sodium, to give products resembling natural rubber. **Gutta-percha** from the latex of various trees of the *Sapotaceæ* from Malay is similarly a polymer, or mixture of polymers, of isoprene. The simplest formula of the many which have been put forward for the rubber hydrocarbon, represents it as a polymer of the following structural unit, in which *n* may be 5 or thereabouts: $[-CH_2{\cdot}C(CH_3){=}CH{\cdot}CH_2-]_n$.

Isoprene, which may be described as a *hemiterpene,* is of fundamental importance, since, as was first suggested by Wallach, all the naturally occurring alicyclic compounds and olefinic terpenes may be looked on as built up structurally from isoprene units. This idea is illustrated in the following structural formulæ for a terpene, a bridged-ring terpene, and a sesquiterpene (bicyclic type):—



(I) Monocyclic nucleus

(II) Para-bridged ring nucleus

(III) Sesquiterpene nucleus (Cadinene type)

FIG. 7.

It is also of interest in plant synthesis that the isoprene nucleus occurs in some of the carotenoids, in the sterols, and in the phytol component of the chlorophyll molecule.

Phytochemical Relationships

The pioneer researches of R. T. Baker and H. G. Smith on *Eucalyptus* and other Australian genera of the *Myrtaceæ* have shown not only that botanically distinct species are distinguished by their chemical constituents, but also that in some cases where morphological examination shows little or no difference, a chemical analysis of the essential oil reveals the existence of completely distinct species. Cases in point are *E. australiana* and *E. phellandra,* the oil from the former containing about 70 per cent. of cineole and little phellandrene, while *E. phellandra*, which is almost indistinguishable morphologically from the first species, yields an oil containing less than 30 per cent. of cineole, and a large amount of phellandrene. These investigators have also advanced a theory of evolution of the various types and species of *Eucalyptus*, in which they attempt to correlate progressive morphological changes with concomitant changes in the chemical character and yield of the essential oils. For instance, *E. corymbosa* has a leaf with an obtuse 'feather' venation, yielding only about 0·5 per cent. of oil, which is mainly pinene; *E. globulus* has a leaf with a more acute lateral venation and a marginal vein, yielding about 2 to 3 per cent. of a pinene-cineole oil; while *E. dives* has the so-called 'butterfly-wing' venation, with increased space for oil glands between the lateral veins, and yields about 4·5 per cent. of a phellandrene-piperitone oil.

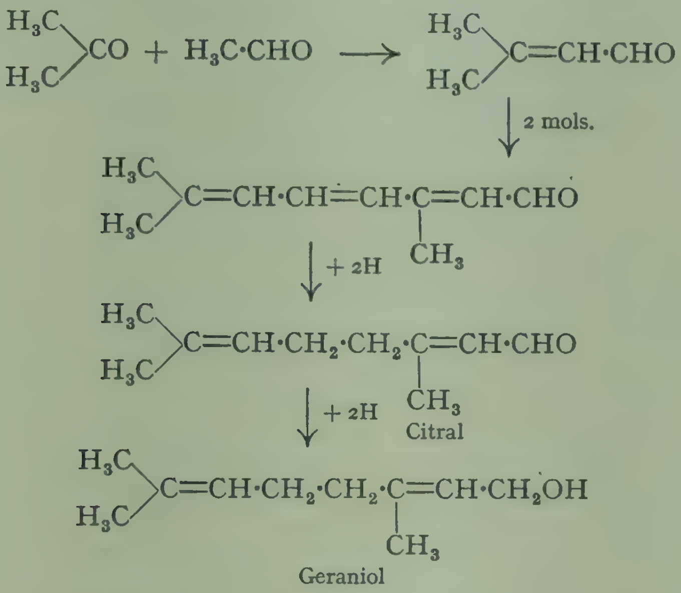
Phytochemical relationships have also been used by Simonsen in the classification of certain Indian grasses, comprising species of *Cymbopogon* and *Andropogon*.

The oil of the same species, however, under different conditions of soil and climate may alter in chemical composition, *e.g.* 'English' lavender grown in France produces an increased amount of linalyl acetate, which rises from 10 to about 35 per cent., and correspondingly less cineole.

Synthesis of Essential Oils in the Plant. Structurally, all the terpene constituents of the essential oils are formed from isoprene nuclei; moreover, treatment of isoprene with acetic acid containing a little sulphuric acid gives a mixture of condensation products including geraniol and α-terpineol. Nevertheless, it is unlikely that isoprene is the precursor of terpenes and camphors in the plant. Read has suggested that the olefinic alcohol, **geraniol,** which is widely distributed in plants, both free and as esters such as the acetate, is the reactive product from which others are derived. Geraniol, in turn, may perhaps originate from simple substances like acetone and acetaldehyde; two successive condensations, followed by reduction, may be pictured as leading to citral, the aldehyde of geraniol (see p. 241).

Geraniol is chosen as the 'key substance' as it possesses a peculiarly reactive grouping (primary alcohol adjacent to a double bond); from it, by simple chemical changes such as ring-closure, addition and removal of water, oxidation, and reduction, most of the naturally occurring alicyclic compounds can theoretically be derived. This can be seen by comparing the potential cyclic formula for geraniol (p. 237) with the formulæ for terpenes, piperitone, etc.

Physiological Function in Plants. The distribution of the essential oils in plants has already been discussed (p. 229). The oil is secreted in various organs such as closed cells, and intercellular spaces or 'oil glands'; it may either remain there, or pass out from the cells into other organs, or diffuse into the atmosphere. In flowers and fruits, the essential oils, like the pigments, may perform the function of attracting insects, etc. In bark, the essential oil may act *protectively against insect attack*, as some of the oils are deterrents or even insecticides: thus, according to H. G. Smith, the timbers of certain species of *Callitris* are immune to the attack of termites owing to the presence of a peculiar phenol to which the odour of the wood is due. In leaves, it has been suggested (Tyndall) that the essential oils *regulate the rate of transpiration*, the slow diffusion of the oil preventing too great a transpiration in the daytime; while at night the oils prevent too great a drop in temperature—as water saturated with oil has a different heat conductivity from water alone. Charabot has shown that the oil content of Peppermint plants varies with the amount of irradiation received by the plant. The composition of the oil of any one species, especially in plants where the oil is secreted in glands, as in many of the *Labiatæ* and *Compositæ*, often varies with the time of year; during the growing season the more volatile components are continually replenished, but in the autumn when synthesis ceases a thick resinous material is left in the glands. Considering the variety of aromatic and alicyclic compounds present in essential oils, many of them are probably by-products in the plant's metabolism.



BALSAMS AND RESINS. DITERPENES

Occurrence and Properties. Occurring with the essential oils in plants, and often isolated with them, are more complex substances called the balsams and resins. The resins must not be confused with the plant gums. The latter are carbohydrates, soluble in water, while the resins are insoluble in water but soluble in the common organic solvents, and usually in the essential oils. A distinction, which is partly one of physical association, is often made as follows: (*a*) the **resins,** which are usually transparent, brittle substances; (*b*) the **gum-resins,** which are plant exudations containing a mixture of true gums, essential oil, and a large amount of resin; and (*c*) the **balsams,** which are similar to the resins and usually obtained by wounding the plant, when the injured cambium layers of cells develop a pathological secretion. Examples of these three groups are given below.

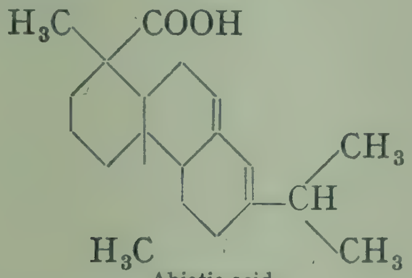
Resins. Rosin, or *colophony*, occurs in solution in crude 'oil of turpentine', and is the residue left after removal of the volatile oils by steam distillation. It is composed mainly of **abietic acid,** which is an isomer of the natural resin acid, *laevo*-**pimaric acid.** The isomerisation consists in a shifting of two double bonds, and is brought about by heating or by treatment with acid. Small amounts of the original *laevo*-pimaric acid can be isolated from Pine exudates; it is significant that less *laevo*-pimaric acid is isolated in summer than in winter, the cell-sap being more acid in summer. Other commercially important resins are **dammar** from species of *Hopea* and *Shorea* in the Malay States; **sandarac** from species of *Callitris*; **dragon's blood** from the Rattan Palm (*Dæmonorops draco*); the fossil resin **amber** (from *Pinites succinifer*); and **copal,** the East African deposits (from *Trachylobium* spp.) and New Zealand Kauri copal (from *Dammara australis*) being fossil, although South American copal comes from the living tree (*Hymenæa*).

Gum-resins. Myrrh from species of *Commiphora* of the *Burseraceæ*, **asafœtida** from species of *Ferula* of the *Umbelliferæ*, and **gamboge** from *Garcinia morella* are the most important.

Balsams. The commonest balsams are **gum benzoïn** from species of *Styrax*, **balsams of Tolu** and **Peru** from the wood and bark of species of *Myroxylon*, **frankincense** from species of *Boswellia* (of the *Burseraceæ*), and **Canada balsam,** which is naturally produced in the schizogenous ducts in the bark of the so-called Balsam Fir (*Abies balsamea*) and allied species.

Structure. Chemically, the resinous matter of all these products consists of substances of high molecular weight, and on degradation by dry distillation or by alkaline fusion they yield aromatic products. In some cases they are *condensation products* of aromatic aldehydes and phenols, while some of them appear to be *polymerisation* and *oxidation* products of the terpenes. For instance, pinene absorbs oxygen and resinifies, and rosin is probably formed in some such reaction. These compounds may be divided into four chemical groups, the **diterpenes,** the **resin acids,** the **resin esters,** and **resenes. Diterpenes** and higher polymers are only slightly volatile in steam. They occur along with the essential oils in balsams and resins. **Camphorene,** $C_{20}H_{32}$, occurs in oil of camphor; it is built up of *four isoprene units.* **Phytol,** $C_{20}H_{39}OH$, is present in the molecules of chlorophyll, vitamin K_1, and the tocopherols (vitamin E). It is a straight-chain alcohol (p. 194) built up of four isoprene units, and therefore belongs to the diterpene group.

Abietic acid, $C_{19}H_{29}COOH$, is the most important resin acid. It also is composed of four isoprene units, and the ring structure is that of a reduced phenanthrene (p. 226).



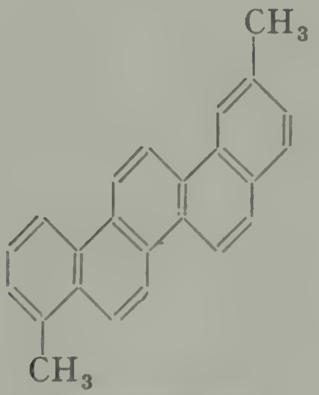
Abietic acid

Certain other naturally occurring resin acids are more stable than *laevo*-pimaric acid and are therefore isolated unchanged from the plant. *Dextro*-**pimaric acid** occurs in 'French galipot', a semi-solid resin from *Pinus maritima.* The name is misleading as it is not a mirror image of turpentine *laevo*-pimaric acid, but a structural isomer. It contains a reduced phenanthrene ring. **Podocarpic acid** is obtained from the resins of *Podocarpus* species. The formula, $C_{16}H_{19}(OH)(COOH)$, contains a reduced phenanthrene ring, and since there are only seventeen carbon atoms, it may be regarded as a degraded diterpene derivative.

Resin esters include simple esters such as **benzyl benzoate,** the main constituent of dragon's blood, **cinnamyl cinnamate** in storax, and esters of both benzoic and cinnamic acids in Peru and Tolu balsams. Asafœtida contains esters of **ferulic acid,** $C_6H_3(OH)(OCH_3)\cdot CH{=}CH\cdot COOH$, and **umbellic acid,** $C_6H_3(OH)_2\cdot CH{=}CH\cdot COOH$, with the anhydride of the latter, **umbelliferone.** More complex esters also occur, but all of them give on hydrolysis with alcoholic potassium hydroxide the *potassium salts of the resin* *acids*, and *alcohols*. The resenes contain oxygen, but are chemically inactive substances; they are probably polymerisation products. Myrrh, dammar, and mastic belong to this group.

TRITERPENES

The triterpenes are widely distributed in plants, and may occur in any part of a plant. They are present both in the free state and combined with sugars. The glycosides form one important group of saponins (p. 109). The fundamental structure is that of **picene,** a conjugated hydrocarbon built up of five condensed benzene rings. Dehydrogenation of the triterpenoid sapogenins gives 1, 8-dimethyl-picene (structurally composed of *six isoprene units*), thus distinguishing them from the sapogenins which give steroids.



1, 8-Dimethyl-picene

- **Ursolic acid,** $C_{30}H_{48}O_3$, is widely distributed as the wax-like coating on leaves and fruits, *e.g.* in Apple and Cherry. It is structurally composed of six isoprene units, and has the carbon skeleton of picene.
- **Oleanolic acid,** $C_{30}H_{48}O_3$, occurs free in the leaves of the Olive, the skin of Grapes, and the buds of Cloves. It is also present as a saponin in the Sugar Beet.
- **Betulin,** $C_{30}H_{50}O_2$, the white pigment of Birch bark (*Betula alba*) also belongs to the triterpenes.
- **α-Amyrin,** $C_{30}H_{50}O$, is a component of resins and of Shea 'butter' (p. 29).

PART VII. PLANT METABOLISM

CHAPTER XXII

ENZYMES

Enzymes are organic colloidal materials elaborated by living organisms, both plant and animal, which act as catalysts in certain organic chemical reactions. Examples of enzymes and enzymatic action have already been encountered in discussing the three main chemical groups of plant materials, *viz.* the carbohydrates, fats, and proteins. We shall see that they are probably essential also for plant respiration and for photosynthesis.

Historical. The use of yeast and malt dates back to early times, and the reactions which they catalysed were termed 'fermentations'. In 1810, Planche showed that extracts of various plant roots contained oxidising enzymes, as they gave a blue colour with guaiacum tincture. This is an alcoholic extract of guaiacum gum, obtained from species of *Guaiacum* trees, which contains guaiaconic acid and yields a blue oxidation product. A little later, several investigators examined the hydrolysis of amygdalin in bitter almonds and isolated *emulsin.* In 1830, Dubrunfaut prepared an extract of malt, and in 1833 Payen and Persoz isolated malt *diastase* by precipitation with alcohol. In 1835, Fauré described the action of *sinigrin* in black mustard seed. The first investigation of the general character of enzymes was carried out by Pasteur, who developed the theory of the specificity of 'ferments.' Buchner, as a result of his work on yeast in 1897, recognised further that enzymatic activity was not dependent on the living cell, but was retained by the expressed juice or extract.

Occurrence and Isolation. Enzymes are present in all living cells of plants, and in greatest variety in seeds. As they are colloidal, they cannot diffuse through the cell-wall, hence most plant enzymes are intracellular. In some cases the enzyme and its substrate (the chemical substance or substances, the reaction of which is catalysed by the enzyme) are in different cells, and enzyme action takes place only after the rupturing of the cell-wall, *e.g.* in glucosidic hydrolysis (p. 102). When enzyme and substrate are in the same cell, the enzyme is probably present for part of the plant's life in some inactive form such as a proenzyme. There are also some extracellular enzymes, especially in lower plant forms such as fungi, which cannot absorb substances like starch until they have been hydrolysed outside the organism. The pepsin-like enzyme of insectivorous plants (p. 159) is another example.

In a few cases, the enzyme can be obtained directly from the plant juice, *e.g.* Pineapple juice yields a protease (*loc. cit.*). Otherwise methods of extraction are used which depend on the rupturing of the cell-wall (*a*) by grinding the plant with sand, either with or without previous freezing or drying of the tissue; or (*b*) by autolysis with toluene or chloroform—neither of which, with a few exceptions, injures the enzymes—followed by extraction of the mass with a solvent. This is usually water, often containing small amounts of acid or alkali to give a definite p_H, or in some cases glycerol. From these extracts the enzyme is precipitated with alcohol, acetone, or ether, or is adsorbed from the solution by an adsorbing agent such as aluminium hydroxide, kaolin, or charcoal. Many enzymes show preferential adsorption at different hydrogen-ion concentrations, and this method is used extensively in the separation of an enzyme mixture.

The exact nature of all enzymes is not yet known. They are colloidal substances, whose catalytic activity per unit weight can be greatly enhanced by purification. Willstätter isolated a peroxidase from Horseradish which was 20,000 times more active per gram of dry weight than the original preparation; it activated 1000 times its weight of hydrogen peroxide per second at 20° C. Several enzymes have been crystallised, *e.g.* urease, papain, and catalase. Some are **proteins,** *e.g.* urease, others are **conjugated proteins** (p. 148) and the prosthetic groups have been isolated and identified as individual chemical compounds. These have been discussed in their proper sections (pp. 163, 170, 198). In such cases the specificity of the enzyme for a particular substrate or substrates resides in the *protein* part of the conjugated system.

Since there is as yet no criterion for the purity of all enzymes, their characterisation and estimation must be based on the reactions which they catalyse. The measurement of enzyme action is therefore made by determining either (*a*) the time required to change unit quantity of the substrate, or (*b*) the quantity of substrate changed in unit time, both measurements being made at specified temperatures and p_H values.

Classification. In Table VI the chief plant enzymes are classified in large groups according to the type of reaction they catalyse. It must be remembered that some of the earlier named enzymes are in reality mixtures, *e.g.* emulsin and zymase. This last 'enzyme' contains at least two phosphatases, a carboxylase, an isomerase, and a dehydrogenase. The first group of enzymes has been discussed in relation to their substrates in the preceding chapters. Before discussing Groups II and III, it seems advisable to consider enzyme action generally as a branch of catalysis.

TABLE VI

Enzyme	Plant sources of enzyme	Substrate	Products
Group I. Hydrolysing Enzymes			
(a) *Enzymes hydrolysing Esters* (*Esterases*).			
Lipase	Oily seeds	Fats (organic esters)	Fatty acids + glycerol
Pectase	Fruits; Clover, etc.	Pectin	Pectic acid + methyl alcohol
Chlorophyllase	Leaves	Chlorophyll	Chlorophyllide + phytol
Phosphatase	Yeast; seeds	Phosphates and their esters	H_3PO_4 + hydroxy compounds
(Phytase)	Seeds	Phytin	H_3PO_4 + inositol
(b) *Enzymes hydrolysing Carbohydrates and Glycosides.*			
α-Glucosidase	Leaves; cereal grain	α-Glucosides	Glucose + hydroxy compounds
(Maltase)	,,	Maltose	2 mols. glucose
β-Glucosidase	Leaves of Cherry, etc.	β-Glucosides	Glucose + hydroxy compounds
(Prunase, emulsin)	,,	Mandelonitrile-glucoside	Glucose + mandelonitrile
Invertase (sucrase)	Leaves; yeast	Sucrose	Glucose + fructose
α-Amylase	Germinating grain	α-Amylose	Maltose
β-Amylase	Ungerminated grain; soya-beans	β-Amylose; dextrins	Maltose + dextrins
Diastase (=α- + β-amylase)	Germinating Barley	Starch; dextrins	Maltose + dextrins
Inulase	Artichokes, Dahlias	Inulin	Fructose
Cellulase	Espec. seeds	Cellulose	Cellobiose
(Cytase)	,,	Hemicelluloses	Reducing sugars
Pectinase	Germinating Barley	Pectin	Reducing sugars
Amygdalase	Almond seeds	Amygdalin	Mandelonitrile-glucoside + glucose
Sinigrinase (myrosin)	*Cruciferæ*	Sinigrin	Allyl *iso*thiocyanate + glucose + $KHSO_4$
Indemulsin	Indigo plants	Indican	Indoxyl + glucose

Table VI (*contd.*)

Enzyme	Plant sources of enzyme	Substrate	Products
(c) *Enzymes hydrolysing the C–N Linkage.*			
Exopeptidases	All plants	Proteins, peptides	Amino-acids
Asparaginase	Germinating Barley	Amides	Amino-acids + NH_3
Endopeptidases	All plants	Proteins	Protein derivatives
(Papain)	Fruit of *Papaya* tree	"	" "
(Bromelin)	Fruit of Pine-apple	"	" "
(Ficin)	Fruit of Fig	"	" "
(Asclepain)	Milkweed	"	" "
Deamidases	All ? plants	Acid amides	Acid + NH_3
Arginase	Seedlings of Vetch, etc.	Arginine	Ornithine + urea
Urease	Soya bean, etc.	Urea	$NH_3 + CO_2$
Transaminase (deaminase)	All plants	Amino-acids	Hydroxy- and keto-acids + NH_3
Group II. Enzymes catalysing Oxidation and Reduction (Oxidases, Reductases, Dehydrogenases)			
(a) *Copper Proteins* (p. 148):			
Monophenol oxidase	Wild mushroom	Phenol	Quinone
Polyphenol oxidase	Cult. mushroom; Spinach	Polyphenols	Quinones
(Catechol oxidase)	"	Catechol	*o*-Quinone
(Tyrosinase)	Potato; Mushroom	Tyrosine	Melanin
Laccase	Lac tree	Urushiol	Black compound
(b) *Hæmoproteins* (p. 198):			
Catalase	All higher plants	H_2O_2 + alcohols	H_2O + aldehydes
Peroxidase	All higher plants	H_2O_2 (peroxides)	H_2O + atomic oxygen
Cytochrome oxidases	Grain embryos	Cytochromes	Oxidised cytochromes
(Indophenol oxidase)	Yeast	"	"
Dihydroxymaleic acid oxidase	Some higher plants	Dihydroxymaleic acid	Maleic acid
(c) *Flavoproteins* (p. 171):			
Warburg's yellow enzyme	Yeast	Other enzymes	
Xanthine oxidase	Lupin	Xanthine	Uric acid
D-Amino-acid oxidase	Some higher plants	Amino-acids	Carboxylic acids + NH_3

TABLE VI (*contd.*)

Enzyme	Plant sources of enzyme	Substrate	Products
(d) *Unclassified*:			
Aldehyde oxidase	Some plants; Potato	Aldehyde + nitrate	Acid + nitrite
(Reducase)	Some plants	Nitrate	Nitrite
Citric acid oxidase	Cucumber seeds	Citric acid	
Oxalic acid oxidase	Orange seeds	Oxalic acid	
Succinoxidase	Some seeds	Succinic acid	Fumaric acid
Fumarase	,,	Fumaric acid	Malic acid
Ascorbic acid oxidase	Pumpkin, Cucumber	Ascorbic acid	Dehydroascorbic acid
Group III. Enzymes attacking the C—C Linkage			
(a) *Nucleotide Proteins* (p. 170):			
Zymase (mixture)	Some fruits, roots; yeast	Hexoses	Ethyl alcohol + CO_2
Decarboxylase	Carrots, Squash, Green peppers	Glutamic acid	Alanine + CO_2
(b) *Diphosphothiamine Proteins* (p. 163):			
Carboxylase	Some plants; yeast	α-Keto-acids	Aldehydes + CO_2
(c) *Unclassified*:			
Oxynitrilase (emulsin)	Leaves of Cherry, etc.	Mandelonitrile	Benzaldehyde + HCN

N.B. Enzymes bracketed in the same line are alternative names. Enzymes bracketed in the following line(s) are *probably* identical with the unbracketed enzyme above.

CATALYSIS AND ENZYMATIC ACTION

In inorganic chemistry, reactions between substances are usually rapid, as they occur in solution and are in most cases between ions. For example, the precipitation of silver chloride on mixing solutions of sodium chloride and silver nitrate is due to the rapid formation of insoluble silver chloride from the silver and chloride ions already present in solution. Most organic compounds, on the other hand, ionise slightly, if at all, and therefore their reactions are slower, and often reversible. This has been shown in ester formation:

$$\text{ethyl alcohol} + \text{acetic acid} \rightleftharpoons \text{ethyl acetate} + \text{water.}$$

In this reaction, a definite equilibrium point is reached, with all four substances present, whether one starts with ethyl alcohol and acetic acid, or with ethyl acetate and water. In such cases, it is possible to alter (*a*) the **velocity of reaction,** even in processes which are normally so slow as to be imperceptible; and (*b*) the **actual position of equilibrium.** This latter factor depends on the concentration of the reactants (law of mass action), and may be altered by changes in pressure and temperature, and by addition or removal of one of the reactants according to this law. In the above instance of ethyl acetate, the removal of water shifts the equilibrium in the direction of ester formation.

With regard to the **velocity** of reaction, according to a rule formulated by van 't Hoff, chemical reactions generally are speeded up two or three times by a temperature increase of 10° C., and this change in the velocity of chemical reactions is important in plant metabolism, as will be seen particularly in the discussion on photosynthesis (p. 270). The other method whereby the velocity of reaction may be altered is by the action of **catalysts.** The most general definition of a catalyst is a *substance which can facilitate a reaction without entering into the final equation, and without supplying any energy.* This includes the subsidiary laws for catalysts:

(i) In a reversible chemical reaction, *e.g.* ester formation, a catalyst alters the velocity of the reaction, but not the position of equilibrium. That is, the catalyst accelerates the reverse reaction to the same extent.

(ii) The amount of the catalyst at the end of the reaction is unchanged, since the catalyst has not added to, nor subtracted from, the energy of the system. Therefore, relatively small amounts of the catalyst are effective.

This definition also includes such cases as the conversion of glucose into ethyl alcohol by zymase, and into lactic acid by the lactic acid ferment; or the hydrolysis of a protein by different catalysts, *e.g.* acid, alkali, pepsin, and trypsin, where in each case the products are different. It is unnecessary to assume that a solution of protein, even when kept indefinitely, would eventually contain all the possible products; for of the several possible reactions only one may be affected by the catalyst, which therefore determines the *direction of the reaction.*

Enzymes as Catalysts. Substances which catalyse reactions among organic compounds may be divided into *inorganic* and *organic catalysts*, with a subdivision of organic catalysts into *non-enzymatic* and *enzymatic.*

Inorganic catalysts, especially the *hydrogen ion*, are common in organic reactions, *e.g.* in the hydrolysis of sucrose or the proteins by hydrochloric acid; and minute and localised alterations in the p_H value in the plant undoubtedly play a large part in bringing about chemical changes. *Metals*, such as platinum and nickel, are used industrially to catalyse organic reactions, *e.g.* nickel is used in the hydrogenation of fats and oils. The importance of traces of such metals as iron, manganese, and copper in plant life, particularly in oxidations, must also be remembered (p. 304); some, if not all, of these metals form part of certain enzyme systems. Non-enzymatic organic catalysts include simple organic substances such as aniline and other bases, which can catalyse decarboxylation, *e.g.* of acetoacetic acid to acetone and carbon dioxide; moreover, complex compounds like glutathione and the cytochromes act as catalysts in oxidative processes in animal cells, and may also play a part in plant respiration (p. 284). In addition, there are the numerous natural organic substances, many of unknown chemical structure, grouped as enzymes.

Enzymes are included in the preceding definition of a catalyst. It must be remembered, however, that the position of equilibrium in a reversible reaction is stable only if the solution remains buffered, in effect only when *true* equilibrium is established, and also only if the products of the reaction have no effect on the enzyme. The hydrolysis of protein by pepsin illustrates this statement, as some of the products ionise in solution, and so the p_H value and therefore the equilibrium of the reaction is altered.

Apart from their source, the chief differences between enzymes and other catalysts are that enzymes are *inactivated by heat* (usually above 60° C.); they require the right *temperature conditions*, and in fact have an optimum temperature for a given reaction (about 40° C.); and finally they are only active within a *definite* p_H *range*. The temperature range for enzyme action is therefore limited, although freezing in most cases merely inhibits the action temporarily, and the enzyme regains its activity when the system is brought back to a higher temperature. It is significant that the temperature range for enzyme activity is much the same as the range for the maintenance of protoplasmic activity, and in fact the ability of the protoplasm to live at high temperatures may be determined by the temperature maxima of its enzymes.

The actual mechanism of enzyme action cannot be fully explained until the structure and molecular size of enzymes themselves are known. There is, however, a *definite association* between the substrate and the enzyme, but this may be either a chemical combination giving a definite compound, or, more simply, adsorption of the substrate on the enzyme surface. It is possible that different enzymes require different types of association with substrates.

Specific Nature of Enzymes. Enzymes are specific either for one compound, or for one grouping or linkage in compounds. The proteases hydrolyse not only proteins, but also many other substances containing a C–N linkage, whereas the hydrolytic action of maltase is confined to the definite stereochemical configuration of α-glucosides. Similarly the prunase of emulsin acts on prunasin, sambunigrin, gentianose, and raffinose, which all contain a β-glucosidic linkage; while zymase ferments glucose, mannose, and fructose, which have a common enol form (p. 68), and not *d*-galactose, which differs in the arrangement of groups on the fourth carbon atom. Lipase hydrolyses almost all ester linkages, but preparations of lipases from different sources bring about different rates of hydrolysis of various types of esters. For example, lipase from *Ricinus* is more effective in the hydrolysis of fats than of esters of the lower fatty acids, while with lipase from *Aspergillus* the reverse is true. So selective may be the action of the enzyme that asymmetric synthesis results, for instance, in the action of lipase from *Aspergillus* on methyl *dl*-mandelate, where the methyl *d*-mandelate is preferentially hydrolysed. This can be explained if the enzyme is itself optically active, and if definite enzyme-substrate compounds are formed, for these compounds would be diastereoisomerides of the type $dAlB$ and $dAdB$; these would have different physical properties and different rates of formation and decomposition, and thus account for the selective hydrolysis of one of the optically active forms of the racemic ester. The recognition of this specificity has induced many investigators to give new names to each enzyme isolated, many of which have afterwards been shown to be identical. The difficulty of freeing the enzyme from impurities will vary with the source, and is apt to create anomalies. For instance, the different values found for the optimum p_H for the action of an enzyme from different sources and on different substrates may be due wholly to this factor. Lipase from *Ricinus* acting on tributyrin has an optimum p_H of 5; the corresponding value for lipase from *Aspergillus* acting on tributyrin is 8·6, while for lipase from *Carica Papaya* acting on olive oil it is 6.

SYNTHESIS BY ENZYMES

Since an enzyme cannot alter the equilibrium of a reaction, it must catalyse the anabolic action as much as the katabolic action, although the latter is the one by which the enzyme is characterised.

Synthesis by enzymes has been achieved for several α-glucosides by using maltase, the first being that of a disaccharide from glucose (Croft Hill, 1898). Later Borsook and Wasteneys (1925) synthesised proteins from peptone, using pepsin at p_H 4; and Blagoveschenski (1926) claimed a synthesis of polypeptides from leucine and glycine, using the protease from seeds of *Phaseolus Mungo*. Asymmetric syntheses of optically active mandelonitriles from hydrocyanic acid and benzaldehyde have been achieved; Almond emulsin gives *d*-mandelonitrile, whereas emulsin from Peach and Cherry gives the *laevo*-isomer. In the living cell there exists a balance between katabolism and anabolism, and there is little doubt that in many reactions the same enzyme under different conditions catalyses both types. On the disorganisation of the protoplasm, however, as in autolysis, only breakdown processes are possible, *e.g.* the liberation of hydrocyanic acid from cyanophoric glucosides. Synthesis by enzymes in the living plant depends to a large extent on factors such as the water content of the cell, especially with hydrolysing enzymes.

Proenzymes, Activators, Coenzymes, and Inhibitors

Apart from the modification of enzymatic reactions by p_H and temperature, the fulfilment of certain other conditions is often necessary to effect maximum activity, or even to give rise to reaction at all.

A **proenzyme,** or zymogen, is an inactive substance from which the enzyme is developed. Sometimes it is a chemical precursor of the enzyme, into which it is converted by another organic substance, by a change in p_H, or by the formation of salts with metals; or, again, the proenzyme may be the enzyme itself in conjunction with an inhibitory substance which is destroyed by an activator. An example of a plant proenzyme is provided by the form of lipase present in ungerminated seed and termed 'spermatolipase.' This is only active in an acid medium not present in the seed; but during germination it changes into 'blastolipase,' which is active in neutral and slightly alkaline solutions.

Activators may be defined as substances which increase or permit the activity of an enzyme, and they are of several kinds. For instance, hydrocyanic acid and sulphuretted hydrogen both activate several plant proteases, especially papain and bromelin (p. 159). Laccase requires the presence of manganese (p. 263) for maximum activity. Again, arsenic activates one only of the phosphatases present in yeast zymase, thus establishing a distinction between these two enzymes. In another type of activation the enzyme preparation can be separated into two or more fractions, all of which are necessary for full activity or sometimes for any activity at all. Phosphate is present in zymase, and is necessary for the functioning of one of the enzymes in this mixture, as it forms a definite intermediate compound in the fermentation process. The term **coenzyme** is usually reserved for the dialysable organic substance which can be separated from the colloidal enzyme system. Neither fraction by itself shows any activity. Such enzymes are probably all *conjugated proteins*, and the coenzymes are therefore the **prosthetic groups.** The best example is *cozymase*, which may be separated from the colloidal enzyme mixture *apozymase* by a special gelatine filter; without cozymase, apozymase is inactive. Apozymase is a mixture of enzymes with various functions which include phosphorylation, isomerisation, and oxidation-reduction (p. 256). The coenzymes which have been purified and identified as chemical compounds of known structure include **cozymase** (coenzyme I) (p. 256), **cocarboxylase** (p. 163), and the **coenzymes of oxidation-reduction,** which are listed in Table VI, p. 248.

An **inhibitor** is a substance which retards or stops the action of an enzyme, so that the effect is comparable with the poisoning of catalysts in inorganic reactions. Sulphuretted hydrogen and hydrocyanic acid inhibit a number of catalyses, including the action of oxidases, zymase, and some proteases; this effect may be due either to their action on metallic ions, which are in some instances part of the enzyme molecule, or to their action on the substrate rather than on the enzyme.

Organic substances may act as inhibitors, *e.g.* acetophenone inhibits the hydrolytic action of lipase on ethyl butyrate, and its oxime does not, hence the inhibiting action is here due to the carbonyl ($-C=O$) grouping. Inhibitors are in some cases chemically related to the substrate, and therefore compete for the enzyme with the substrate, so that the decomposition of the latter is retarded. Or again, the inhibitor may be one of the products of the reaction; for instance, β-glucose inhibits the hydrolysis of sucrose by invertase of yeast. This type of inhibition, which is distinct from the reversibility of the reaction since the equilibrium position is near complete hydrolysis, is probably another essential factor in the regulation of many reactions in the living cell, for instance in the carbohydrate balance in the green leaf.

ALCOHOLIC FERMENTATION

Of the enzymes listed under Group III on p. 249, the zymase complex is the most important, because of (*a*) the economic importance of alcoholic fermentation, (*b*) the widespread occurrence of the constituent enzymes in the higher as well as in lower plant forms, and (*c*) the close relation between fermentation and respiration (p. 280).

The nett chemical change in fermentation is the decomposition of hexose to ethyl alcohol and carbon dioxide:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Various intermediate products have, however, been identified, both directly and by the addition of substances which lead to their accumulation. Hence the reaction is complex, passing through definite stages, which are catalysed by the various enzymes of the zymase complex.

Neuberg, Meyerhof, and other workers have shown that the following transformations occur:

Hexose → fructosediphosphate → pyruvic acid →
acetaldehyde → ethyl alcohol.

Stage I. Hexose → fructosediphosphate

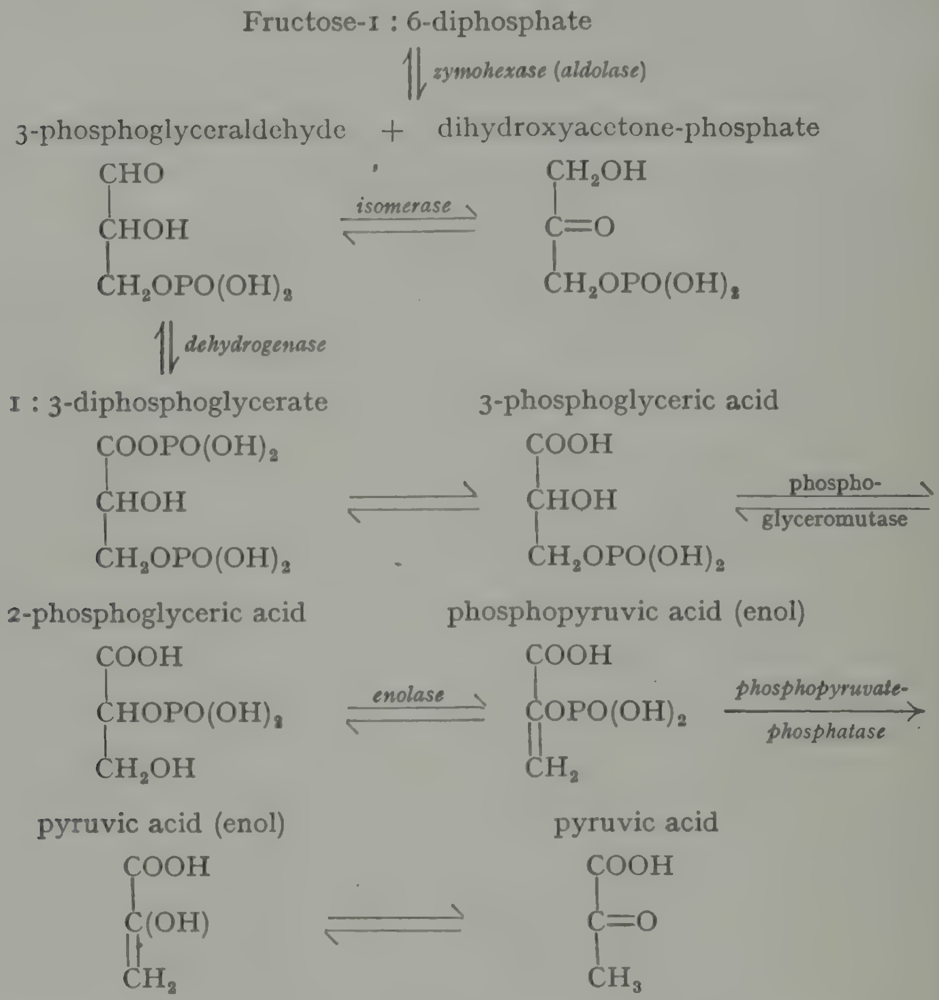
It can be shown that the addition of inorganic phosphate accelerates the fermenting action of dried yeast or yeast juice; that it does not accelerate the action of living yeast cells is due to the occurrence of sufficient organically combined phosphate in the cells. The action of the phosphate is to form an ester-like compound, a hexosephosphate, and the same compound, *viz.* fructofuranose-1 : 6-diphosphoric acid, is isolated in quantity whether glucose, fructose, or mannose is the sugar fermented. These three hexoses possess a common enolic form (p. 68), whereas galactose, which is not fermented, gives a different enol; hence enol formation must be the precursor of the phosphate ester. Three steps in the formation of the diphosphate have been identified, each catalysed by a specific enzyme:

$$\text{Hexose} \xrightarrow{\textit{hexokinase}} \underset{\text{(Robison ester)}}{\text{glucose-6-phosphate}} \xrightarrow{\textit{isomerase}} \underset{\text{(Neuberg ester)}}{\text{fructose-6-phosphate}} \xrightarrow{\textit{phosphohexokinase}} \text{fructose-1 : 6-diphosphate.}$$

The two hexokinase (or phosphorylase) enzymes contain prosthetic groups belonging to the *adenosine triphosphate* system (ATP) (p. 170), donating phosphate and being converted to the diphosphate (ADP). Isomerase appears to be a *metallo-protein.*

Stage II. Fructosediphosphate ⟶ pyruvic acid

Here six enzyme-controlled transformations result in the formation of a three-carbon compound by splitting the hexose molecule and removal of phosphate:



The dehydrogenase was originally known as Warburg-Christian's 'ferment', and its coenzyme is the *diphosphopyridine nucleotide* (DPN) known as *cozymase* or coenzyme I. The coenzyme of the phosphopyruvate-phosphatase belongs to the *adenosine diphosphate* type (ADP), accepting phosphate to become a triphosphate. This is the reverse of the phosphorylation in Stage I. The removal of the labile phosphate from the carboxyl group of 1 : 3-diphosphoglycerate is probably a simple transference to adenylic acid with the re-formation of adenosine triphosphate. Zymohexase, isomerase, and enolase are *metalloproteins*; in enolase the metal is magnesium, and this appears to be necessary for the phosphatase also. That the formation of pyruvic acid is a definite stage in the fermentation reaction has been shown by the isolation of a condensation product of pyruvic acid and β-naphthylamine on the addition of the latter to an alcoholic fermentation. The addition of sodium fluoride stops the reaction one step earlier and results in the accumulation of phosphopyruvic acid.

Stage III. Pyruvic acid ⟶ acetaldehyde

Pyruvic acid is decomposed to acetaldehyde and carbon dioxide by the action of the enzyme *carboxylase,* which acts generally on α-keto-acids with the liberation of carbon dioxide:

$$CH_3{\cdot}CO{\cdot}COOH \longrightarrow CH_3{\cdot}CHO + CO_2$$

Carboxylase has been isolated from yeast and many higher plants, *e.g.* Onion, Spinach, and Orange. It is a *conjugated protein,* and the prosthetic group in yeast carboxylase, *i.e.* yeast cocarboxylase, is *diphosphothiamine hydrochloride* (p. 163). The enzyme also requires magnesium for its action. The presence of acetaldehyde can be shown in fermentations which are kept anaerobic, so that there is no possibility of the acetaldehyde being produced by the atmospheric oxidation of preformed ethyl alcohol. The aldehyde can be isolated as a condensation product with dimethyl-*cyclo*-hexanedione (dimedone).

Stage IV. Acetaldehyde ⟶ ethyl alcohol

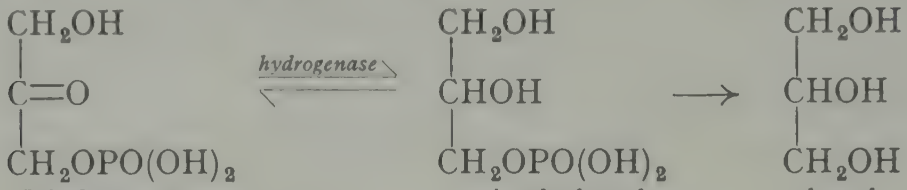
The final stage is the reduction of acetaldehyde to ethyl alcohol:

$$CH_3{\cdot}CHO \underset{-H_2}{\overset{+H_2}{\rightleftharpoons}} CH_3{\cdot}CH_2OH$$

This reaction is controlled by Warburg's 'reducing enzyme of fermentation', which contains *cozymase* as its prosthetic group. The nicotinamide portion of the molecule is responsible for the hydrogen transfer. This step may be coupled with the oxidation of 3-phosphoglyceraldehyde in Stage II, so that the coupled reaction may be written:

$$\begin{aligned}&CH_3{\cdot}CHO + CHO{\cdot}CHOH{\cdot}CH_2OPO(OH)_2 \rightleftharpoons \\ &\qquad CH_3{\cdot}CH_2OH + COOH{\cdot}CHOH{\cdot}CH_2OPO(OH)_2\end{aligned}$$

A modification of the fermentation reaction occurs when the reduction of acetaldehyde to ethyl alcohol (Stage IV) is prevented by forming the acetaldehyde-bisulphite or other additive compound. Then the equilibrium of the mixture of 3-phosphoglyceraldehyde and dihydroxyacetone phosphate (Stage II) is disturbed, and the latter compound is reduced and dephosphorylated by the enzyme system with the formation of glycerol:



$$\begin{array}{c} CH_2OH \\ | \\ C{=}O \\ | \\ CH_2OPO(OH)_2 \end{array} \overset{hydrogenase}{\rightleftharpoons} \begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OPO(OH)_2 \end{array} \longrightarrow \begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

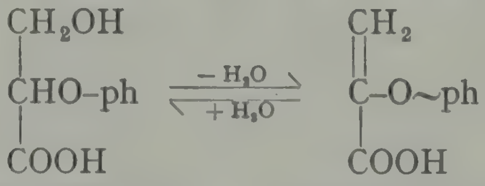
Dihydroxyacetone-phosphate 3-phosphoglycerol glycerol

Neuberg called this the **Second Form** of fermentation; it can be used for the large-scale production of glycerol. The so-called Third Form of fermentation, *viz.* the accumulation of glycerol on the addition of alkalis or alkaline salts, is also explained by a shift in the equilibrium of Stage II as above. Other fermentations, occurring especially in the action of various bacteria on sugars, *e.g.* the formation of butyric and lactic acids, are again modifications of alcoholic fermentation in which the reactants of Stage II take part in other reactions.

Finally, in respiration, the first part of the reaction, termed the 'anaerobic stage' in respiration, is closely parallel to, if not identical with, the fermentation of hexose as far as the production of the three-carbon units, and in some cases as far as acetyl formation. The important modification, the Krebs carboxylic acid cycle, showing the mode of formation of citric, malic, and related acids in plant and animal respiration will be treated in detail in Chapter XXIV.

Energy-Rich Phosphate Bonds

In the fermentation process, phosphate is added to, and removed from, compounds through the agency of the adenylic acid system. It has been recognised that two types of phosphate ester linkages can occur. In the first of these, hydrolysis of the phosphate takes place with the release of only a small amount of energy, whereas in the second, removal of the phosphate is accompanied by the release of a large amount of energy. Such a high energy phosphate linkage is indicated by **∼ph.** In the following example,



$$\begin{array}{c} CH_2OH \\ | \\ CHO\text{–}ph \\ | \\ COOH \end{array} \underset{+H_2O}{\overset{-H_2O}{\rightleftharpoons}} \begin{array}{c} CH_2 \\ \| \\ C\text{–}O{\sim}ph \\ | \\ COOH \end{array}$$

2-Phosphoglyceric acid Phosphopyruvic acid (enol)

the two compounds have a total energy content differing only by the amount due to the water of hydrolysis; but hydrolysis of the enol form releases a much larger amount of energy than does that of the primary alcoholic form.

This is an example of a general type of **high energy phosphate,** *viz.* the acidic enol grouping, $>\mathrm{C{=}C{-}O{\sim}ph}$. Other groupings containing phosphate with a high energy of release are anhydrisation between two phosphates, $\geqslant\mathrm{P{-}O{\sim}ph}$ (as in adenosine triphosphate), anhydrisation between carboxyl and phosphate groups, $>\mathrm{O{=}C{-}O{\sim}ph}$ (as in acetyl phosphate, p. 282), and the nitrogen-phosphate linkage, $>\mathrm{N{\sim}ph}$ (as in arginine phosphate, guanidine phosphate, and creatine phosphate). This last group is important in the animal system, as creatine phosphate furnishes the energy for muscular contraction.

The fermentation reaction, then, in its application to respiration, is primarily a conversion of energy derived from the partial katabolism of carbohydrates into high energy phosphate. This $\sim\mathrm{ph}$ is distributed by the cell catalysts with two ultimate results; (*a*) the release of phosphate, and of energy for respiration, and (*b*) the transfer of energy to anabolic processes such as protein and fat synthesis.

Enzymes Catalysing Oxidation and Reduction

Any attempt to discuss the function of this group of enzymes in plant metabolism calls for a study of the various theories of the mechanism of respiration. For the present, only the classification of these enzymes, their distribution, and the type of reaction they catalyse will be considered. In addition to their possible function in the respiratory process, these enzymes are concerned in the discolouring of plant tissues through the formation of *coloured oxidation products* from some of the cell constituents, after injury or on the death of the tissue; for example, in the browning of many flower petals, of cut apples, and in the browning and blackening of cut tubers of Potato, and of Beet and Mangold roots. It must be remembered, however, that there are some cases in which this development of colour is due to the action of a *hydrolysing* enzyme, which liberates a chromogen from its glucoside; this chromogen may be autoxidisable in air (*i.e.* requires no enzyme to catalyse its oxidation). Instances of this kind are the production of the blue pigment **dipsacotin** from the Teasel (*Dipsacus*), and of the chromogen **hermidin** from *Mercurialis*, which gives two oxidation products, an unstable blue compound, cyanohermidon, and a more highly oxidised yellow pigment, chrysohermidon.

In many enzymatic oxidations there is simultaneous reduction of another molecule, and there may thus be activation of this reaction by another enzyme, giving an *oxido- reductase system* (or *redox*). Many organic compounds are oxidised not by addition of oxygen, but by the *removal of hydrogen, e.g.* a phenol to a quinone. The enzyme activating this type of change is called a **dehydrogenase** or **dehydrase,** and the compound is termed a **hydrogen donor.** In such a reaction the hydrogen may either combine with *oxygen*, forming water or hydrogen peroxide, or with a *compound capable of being reduced*; in the former instance, the oxygen, in the latter, the compound, acts as a **hydrogen acceptor.** An enzyme catalysing this part of the reaction is termed a **reductase** or **reducase.**

The oxidising enzymes may therefore be classified under two heads, as follows:—

(*a*) Enzymes using oxygen as the hydrogen acceptor. They are usually termed **direct oxidases,** and such systems will blue guaiacum without the addition of peroxide.

(*b*) Enzymes using some easily reduced compound, *e.g.* quinone, as the hydrogen acceptor. These are most correctly termed **oxido-reductases,** though often the names dehydrogenase, dehydrase, reductase, and reducase are used for the whole system. The test for such a reaction in a plant tissue is the conversion of the dye methylene blue into a leuco-compound (*i.e.* the dye acts as hydrogen acceptor). According to Bach some of these reactions catalysed by an oxido-reductase can be explained simply by the splitting of water molecules, the hydrogen being taken up by the hydrogen acceptor, and the oxygen by the oxygen acceptor.

Pugh and Raper suggested a similar division of oxidising enzymes into *aerobic* and *anaerobic oxidases*, depending on whether oxygen acts as the hydrogen acceptor or not. Some of these enzymes, however, can act both as aerobic and anaerobic oxidases *in vitro*, *e.g.* the **xanthine oxidase** of milk, which oxidises xanthine to uric acid, may function with either oxygen or methylene blue as the hydrogen acceptor. A similar xanthine oxidase has been isolated from some plants, especially Lupin seedlings.

In Table VI on p. 248 are listed the main types of oxidising enzymes. Catalase, peroxidase, tyrosinase (Onslow's oxygenase), ascorbic acid oxidase, and the cytochrome oxidases are all protein derivatives.

Peroxidase from Horseradish roots (its main source) contains two enzymes, paraperoxidase and peroxidase. The latter has been crystallised, and is a *hæmoprotein* (p. 148), the iron being trivalent. Peroxidase is a secondary oxidation catalyst. It acts on *hydrogen peroxide* formed by several primary oxidising systems, *e.g.* xanthine oxidase, to give water and *atomic oxygen*, rendering the latter available for the oxidation of other substances in the cell:

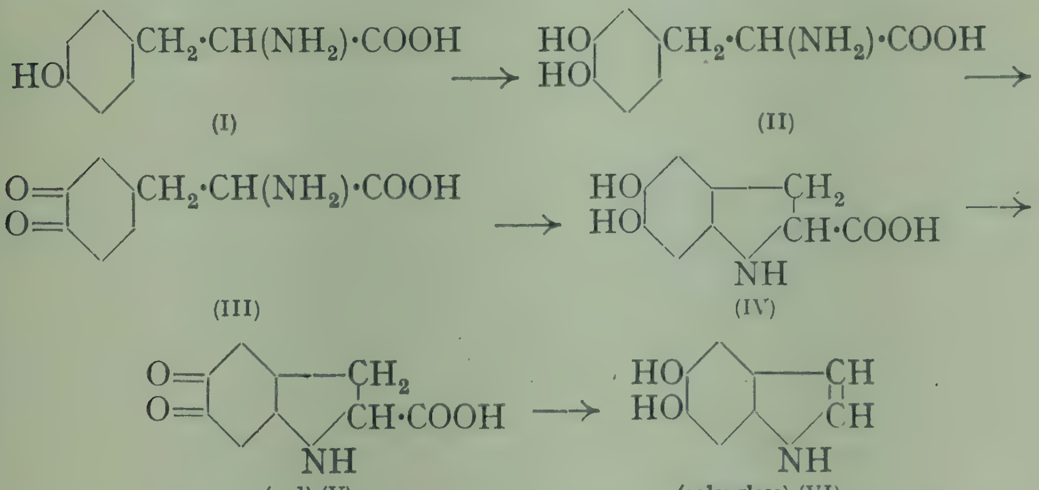
$$H_2O_2 \longrightarrow H_2O + O \text{ (atomic)}$$

Peroxidase may also act similarly on organic peroxides, the formation of which from unsaturated compounds, *e.g.* the unsaturated fatty acids and some terpenes, is a possible intermediate step in their metabolism. A test for peroxidase in a plant tissue is afforded by the blue colour developed in guaiacum tincture *on the addition of hydrogen peroxide.*

Catalase has also been crystallised, and it is a *hæmoprotein.* The prosthetic group is ferrous protoporphyrin IX (p. 198). Catalase is present in most living tissues, and in plants it is found especially in sprouting seeds. Different enzymes with catalase activity have been isolated from different species. In any *one species,* the *protein* is always the *same,* but the number of iron-porphyrin prosthetic groups appears to differ. Catalase also acts on hydrogen peroxide, and it has two functions in the cell. It has a *peroxidase function,* in that it decomposes hydrogen peroxide with the simultaneous oxidation of alcohols to aldehydes, but (unlike peroxidase) it can also decompose excess hydrogen peroxide to water and *molecular* oxygen.

Direct Oxidases

Tyrosinase is the name given to the enzyme isolated from Potato and Mangold which causes the darkening of cut tissues by catalysing the oxidation of the hydroxy-amino-acid *tyrosine* to a black compound *melanin.* An intermediate stage can be distinguished, when a red compound is produced temporarily, changing through a colourless substance to melanin. The actual chemical changes, which have been identified by Raper, entail the introduction of another hydroxyl group (II), the oxidation of this substance to a quinone (III), an intramolecular rearrangement to a dihydroxyindole (IV), followed by oxidation to a quinone-indole (V), which is



the red pigment. This loses carbon dioxide and rearranges to a dihydroxy-indole (colourless) (VI), which in the presence of atmospheric oxygen is oxidised to melanin.

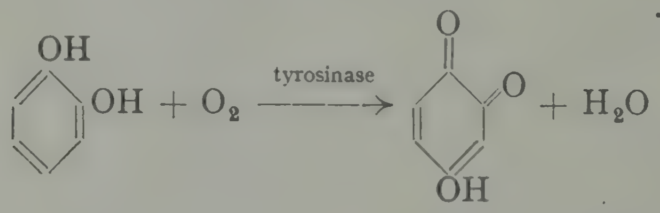
Tyrosinase has also been isolated from many other plants, *e.g.* Wheat, Banana, Apple, Tea, Tobacco, and the cultivated Mushroom (*Psalliota campestris*), and is probably identical with the direct oxidase of plants designated **oxygenase** by Onslow. Tyrosinase preparations can oxidise both *phenol* itself and *polyhydric phenols*, *e.g.* catechol; but on purification, the polyphenol oxidase activity is enhanced. It appears, however, to be a single enzyme, and has been identified as a *copper-protein* complex. **Polyphenol oxidase** action is shown in plants by the blue colour developed with an alcoholic solution of guaiacum. Onslow found it in all the grasses (*Gramineæ*), all the *Compositæ* and *Umbelliferæ*, and in some of the *Leguminosæ*.

Expt. 79. *To test for Polyphenol oxidase and Peroxidase in Plants*

1. Pound the tissue in a mortar with a little distilled water; after a time tissues containing polyphenol oxidase develop a brown coloration.
2. Cut the tissue in small pieces, and add a few drops of chloroform; polyphenol oxidase plants turn brown when kept.
3. Pound the tissue in a mortar with a little distilled water, and add a few drops of an alcoholic solution of guaiacum. If a blue colour develops before ten minutes, polyphenol oxidase is present. To those tissues which give no colour, add a few c.c. of hydrogen peroxide; a blue colour will be developed, showing the presence of peroxidase.

[Suitable polyphenol oxidase tissues are fresh tubers of Potato, fruit of Pear, any part of the Dandelion. Peroxidase tissues are roots of Horseradish and Turnip, and any part of the Wallflower and Pea.]

The action of tyrosinase on *monohydric phenols* is to form the *o*-dihydric phenol or *catechol* (p. 176), whereas the action on *polyhydric phenols* is to form a *hydroxy-quinone*:



Catechol 4-hydroxy-1, 2-quinone

Measurements of tyrosinase activity are usually carried out on catechol or *p*-cresol, and the oxygen absorption measured. But in *plant respiration*, the function of tyrosinase appears to lie in its action on *tyrosine*. Oxidation of tyrosine forms irreversibly the corresponding catechol derivative, **dihydroxyphenylalanine** (formula

II, p. 261). This compound, 'dopa', is then reversibly oxidised to its *o*-quinone (III), and thereby acts as an **oxygen carrier** in the presence of other parts of the respiratory system which are hydrogen donors (*e.g.* ascorbic acid). A trace of tyrosine therefore gives rise to dihydroxyphenylalanine, which then with tyrosinase forms the oxygen-carrying system. Only on injury to the cell with the resultant destruction of the respiratory system does the *o*-quinone (III) continue to accumulate, and then the melanin pigments are formed:

$$\begin{tikzcd} \text{Tyrosine} + O_2 \arrow[r, "\text{tyrosinase}"] & \text{'dopa' (II)} \arrow[r, shift left, "\text{tyrosinase} + O_2"] & o\text{-quinone (III)} \arrow[l, shift left, "\text{hydrogen donor}"] \arrow[d] \\ & & \text{melanin} \end{tikzcd}$$

A similar *copper-protein* oxidase, **laccase,** is obtained from the Japanese Lac tree (*Rhus vernicifera*) and the Indo-Chinese Lac tree (*Rhus succedanea*). Laccase causes the latex of the tree to darken in air with the production of a black, shiny surface, a change which is the basis of the Chinese and Japanese lacquer industry. From the latex the phenolic substances, *urushiol* and *laccol*, which contain the *o*-dihydroxy grouping, have been isolated. The enzyme can also catalyse the aerobic oxidation of other polyhydric phenols and related compounds.

Ascorbic acid (p. 70) is normally present in plant tissues in the reduced form, but when these are damaged it is rapidly oxidised. A specific **oxidase** has been isolated from several plants; it appears to require copper, but as it has been prepared free from traces of copper, it is not a copper-protein like tyrosinase.

The **cytochrome** system contains *iron-porphyrins* characterised by their different absorption spectra. Keilin isolated from yeast three forms, *a*, *b*, and *c*, but some of these are mixtures. Some of the cytochromes are autoxidisable, others require an activating enzyme. Bhagvat showed that cytochromes were present in all higher plant tissues examined. **Cytochrome oxidases** have been isolated from Maize and Wheat embryos, and from Lily pollen. A cytochrome oxidase is also present in the Tea plant. Pigmentation following injury gives rise to 'black tea', whereas inhibition of enzyme activity by steaming the leaves is the method of preparing 'green tea'. Cytochrome oxidases in higher plants are less sensitive to hydrocyanic acid than the corresponding animal enzymes. Keilin isolated from yeast a direct oxidase which was called **indophenol oxidase,** because it oxidised a mixture of *p*-phenylenediamine and α-naphthol to give a dye, *indophenol blue*, which is deposited in the cell, and by which the enzyme is characterised. It is in fact a cytochrome oxidase, since it acts as an activator of atmospheric oxygen for the oxidation of cytochromes *a* and *c* in the respiratory mechanism of the yeast cell. It appears to be identical with the 'respiratory enzyme' of Warburg.

Oxido-Reductases

Mention has already been made of several of these enzymes, *viz. xanthine oxidase* and the dehydrogenase in the fermentation process (p. 256). The mechanism of such reactions has already been discussed (p. 259).

One of the common oxido-reductases in plants is that which oxidises *aldehydes*, *e.g.* acetaldehyde, to *acids*, with a simultaneous reduction of *nitrate* to *nitrite*. It has been detected in Wheat, Flax, Pea, Clover, and Radish, particularly in the shoots, but its main source is the Potato tuber. It is therefore usually called the potato **aldehyde oxidase.** It is specific for aldehydes, some nine of such compounds being possible substrates; the hydrogen acceptor can be nitrate, quinone, or various indicators, including methylene blue.

Eckerson has shown that a similar enzyme, **reducase,** which reduces nitrate to nitrite in the presence of glucose in alkaline solution, occurs in a variety of plants, *e.g.* Apple, Peach, Tomato, Asparagus, Cabbage, Lettuce, Wheat, Soya Bean, Beet. She found that each genus was characterised by a definite distribution of reducase in the various tissues. In the Tomato it was equally distributed in roots and aerial parts; in the Soya Bean it occurred chiefly in the tops; whereas in the Apple it was mainly in the roots, except in early spring, when it occurred for a short time, and in greater amount than in the roots, in the swelling buds. The synthesis of reducase in the plant appears to be contingent upon a supply of both phosphate and potassium.

Other plant oxido-reductases have been described by Thunberg, who used methylene blue as the hydrogen acceptor. He found an **oxalic acid oxidase** in the seeds of the Orange (*Citrus Aurantium*), Plum (*Prunus domestica*) and Mallow (*Malva crispa*); a **citric acid oxidase** in Cucumber seeds (*Cucumis sativus*); and, in the endosperm of the Bean (*Phaseolus vulgaris*), an enzyme which would oxidise various organic acids, *e.g.* malic and succinic acids. A **succinoxidase** which oxidises succinic acid to fumaric acid, and a **fumarase** which oxidises fumaric to malic acid, have also been characterised.

CHAPTER XXIII

PHOTOSYNTHESIS

The Nature of Photosynthesis

PHOTOSYNTHESIS is the name given to the process whereby plants, through utilising *solar energy* absorbed by *chlorophyll*, build up from *carbon dioxide* and *water* various complex organic compounds: these furnish the material of the plant's structure, provide a substrate for respiration and therefore energy for the plant's vital activities, comprise the food storage for the early stages of the next generation of plants, and function as the ultimate source of all the energy which maintains animal life. Priestley (1774) was the first to show that plants evolve oxygen; Ingen-Housz (1779) showed the difference between photosynthesis and respiration; while Senebier (*c.* 1782) demonstrated the importance of chlorophyll, and showed that it was the light—and not the heat—of the sun that was essential for photosynthesis. Later, de Saussure (1804) established a quantitative relationship between the carbon dioxide absorbed and the oxygen evolved, and Sachs (1862) showed that starch was the first 'visible product' of photosynthesis in the chloroplasts.

Photosynthesis is only one of the major activities of the green leaf. Concomitant with it are *respiration*, and the *synthesis* of other organic compounds, such as proteins and fats from the products of photosynthesis. In addition, materials are continuously being translocated from the leaves to other parts of the plant, and therefore it has not yet been possible to determine the first organic product formed in the chloroplasts. Photosynthesis itself comprises a chain of processes: these include the diffusion of carbon dioxide through the stomata (a physical process); the absorption of light and the utilisation of its energy (a photochemical reaction); and, as will be shown later, another reaction, apparently chemical, which is independent of light.

A study of photosynthesis necessitates an investigation of the effect of various factors on the amount of **rate of photosynthesis.** This can be measured by determining: (i) the amount of oxygen liberated per unit area, or per unit of dry or fresh weight of leaf; (ii) the amount of carbon dioxide absorbed per unit area or per unit weight; (iii) the increase in dry weight (Sach's method). Of these,

S

the second method gives the most consistent results, especially if these are calculated on the area of leaf illuminated. For simple water plants such as algæ, the absorption per unit weight is the usual criterion. In the first and second methods correction must be made for respiration, in which process oxygen is absorbed and carbon dioxide evolved. This is usually done by taking measurements for the plant when it is in the dark and therefore only respiring, and then repeating the measurements for the illuminated plant, when both photosynthesis and respiration are taking place. In such calculations it is assumed with little justification that the rate of respiration is the same in darkness as in light.

The factors which must be taken into account include the initial materials carbon dioxide and water, the amount and function of chlorophyll, the effect of the products of the reaction, the temperature, and the intensity and quality of the light.

Limiting Factors. When a reaction depends on several factors, we must take into consideration what Blackman called the *limiting factor.* Liebig, in studying the effect of various plant nutrients on crop yields, was the first to emphasise in his **law of the minimum** that *if one factor is wanting, or at a low level, then this lack limits the effect of all the other factors.* For instance, if phosphates are lacking in the soil, nitrogenous applications will not increase the crop yield beyond a certain value; and the combined application of phosphates and nitrogen (as nitrates, etc.) will give a larger increase in yield than the sum of the increases obtained from either separately. Blackman reformulated this theory, and showed its importance in the measurement of photosynthesis. It may be stated as follows: *When the rate of any physiological process is conditioned by a number of separate factors, then the rate is determined or limited by the magnitude of that factor which is at a minimum.* Many experiments on the rate of photosynthesis under different conditions have shown the fundamental truth of this law, and they also indicate that in photosynthesis a reaction takes place which is independent of light. If the separate factors including the reaction of the plant were independent variables, the graphs obtained for the change in rate with alteration in one factor would show a sharp break and then remain horizontal. In photosynthesis, however, the factors are *interdependent,* and hence the limiting effect of the minimal factor is not absolute but relative. Thus, the curves are logarithmic in form, and show no sharp break; and although there is a slowing down, there is no absolute checking of the effect of the increasing factor on the rate. The following example will make this clear. It is taken from experiments by Lundegårdh on *Oxalis* leaves, and the curves (fig. 8) are composed from his figures. He measured the rate of photosynthesis (by noting the amount of carbon dioxide absorbed per 50 sq. cm. per hour) with increasing concentration of carbon dioxide at a low light intensity (one-fortieth that of direct sunlight). After showing an initial rapid increase, the rate slowed down to an almost constant value. If a higher light-intensity was used, the rate increased more rapidly, finally reaching a higher value, again practically constant. At a still higher light intensity, a still greater rate of photosynthesis was obtained. Hence light was the limiting factor in the first and second instances.

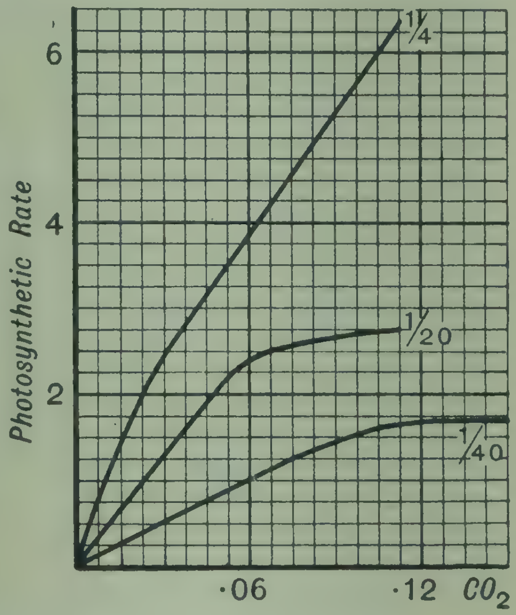


FIG. 8. The Effect of Carbon Dioxide Concentration on the Rate of Photosynthesis at three levels of Light Intensity (after Lundegårdh).

Carbon Dioxide, Water, and the Effects of the Products of Photosynthesis. In the higher land plants the absorption of carbon dioxide is effected by a process of diffusion through the *stomata* into the intercellular spaces of the leaf. In most plants the stomata are more abundant on the under-sides of the leaves than on the upper, and hence the absorption of carbon dioxide is greater on the lower surface. The absorption of carbon dioxide, and also the evolution of carbon dioxide in respiration, on each side of a leaf, have been shown to be directly proportional to the number of stomatal openings present. Brown and Escombe were the first to show that the rate of diffusion of a gas into *small* openings is a function of their diameters, and that with a surface such as a leaf containing a large number of openings, the diffusion of carbon dioxide is relatively more rapid than it would be if the whole area were an absorbing surface. These investigators also measured the absorption of carbon dioxide, and showed that it averaged, for leaves of *Catalpa bignonioides*, 0·07 c.c. at N.T.P. per sq. cm. of leaf surface per hour. Since the stomata on this leaf occupy about 0·9 per cent. of the total leaf surface, the absorption was 7·77 c.c. of carbon dioxide per sq. cm. of stomata per hour. In the case of undifferentiated aquatic plants, such as the algæ, it is possible to measure the effects of supplying varying concentrations of carbon dioxide without complications due to other factors. Warburg conducted classical experiments of this kind on *Chlorella* and showed that at higher concentrations, the assimilation of carbon dioxide increased up to a certain point, beyond which a further increase in concentration did not affect the rate. Hence some other factor—probably the rate of absorption of carbon dioxide at a surface—determined the limitation. The effect exerted on photosynthesis in higher land plants by an increasing carbon dioxide concentration has already been shown (fig. 8). Many other experiments have been carried out at constant higher light intensities. In general, with bright sunlight, average temperature (20°–25° C.), and ample water supply, the carbon dioxide concentration of the atmosphere (3 parts in 10,000) is the limiting factor, and doubling the concentration practically doubles the rate of photosynthesis. This conclusion is of importance in agriculture and horticulture. The rapid development of plants grown in hot-beds prepared by underlaying the soil with manure is probably due both to the higher temperature and to the higher concentration of carbon dioxide present. Experiments have been conducted in various countries on the enrichment of the carbon dioxide content of the air in glass-houses, and positive results have been obtained, although many other factors tend to intervene. Attempts have also been made in Germany to utilise the carbon dioxide in flue gases from blast furnaces, by pumping the gases on to the fields.

The fate of the carbon dioxide after it reaches the intercellular spaces is not clear. Ordinary solution could not account for the very rapid absorption that takes place, and, as we have seen, quantitative measurements indicate the functioning of some adsorbing surface or of some chemical reaction. It is well known that *potassium* is necessary for photosynthesis; its most likely function is combination with carbon dioxide to give bicarbonate. Some such explanation is particularly necessary in the case of such leaves as Sunflower and Nettle, which have a very high rate of carbon dioxide absorption. The absorption of carbon dioxide by higher land plants is complicated by the opening and closing of the stomata and the control of this movement by other factors, such as light, water content, and the accumulation of the products of photosynthesis. The diurnal rhythm in the opening of the stomata is well known, and is principally dependent on light. Seasonal fluctuations also occur; the greatest duration of stomatal opening and the highest photosynthetic rate are in the summer months (July to September). Again, the closing of the stomata is induced by lack of moisture, and by cold (which has probably the same effect, see p. 317). The presence of water also affects the conversion of starch into soluble carbohydrates; moreover, the removal of starch from the stomatal guard cells is one of the causes of the change in osmotic pressure in the latter; this change, in turn, regulates the opening of the stomata. The accumulation of the products of photosynthesis conditions other effects besides the regulation of stomatal opening; nevertheless, the rate of photosynthesis in leaves which are detached does not differ widely, under carefully controlled conditions, from that of leaves attached to the plant.

Chlorophyll. Chlorophyll acts as the transformer of light energy to the chemical energy necessary for the reduction of carbonic acid, H_2CO_3, to carbohydrate $(CH_2O)_x$. The possibility that the chlorophyll molecule may also enter chemically into the photosynthetic process will be discussed later. Chlorophyll is present in the chloroplasts associated with the stroma protein, probably as a conjugated protein, to which the name chloroplastin has been given (p. 148). Willstätter and Stoll showed that under normal conditions the rate of photosynthesis was independent of the amount of chlorophyll in the leaf, or, in other words, that chlorophyll is not usually the limiting factor. They also showed, however, that leaves rich in chlorophyll give a much higher increase in the rate of photosynthesis with increase in temperature than do leaves poor in chlorophyll. The explanation is that in the latter case there is not enough chlorophyll to absorb the greater amount of radiant energy required to allow the higher temperatures to exert their full effect. Again, Emerson working with *Chlorella* found that with a high concentration of carbon dioxide and high light intensity, the rate of photosynthesis was a linear function of the chlorophyll content.

Light Intensity and Temperature. Not all the solar radiant energy incident on the surface of a leaf is absorbed by it; measurements have shown that about 30 per cent. is transmitted, and about 19 per cent. re-radiated. The remaining 51 per cent. is absorbed by the leaf and is used in two main functions, *viz.* vaporisation of water or transpiration (50 per cent.), and photosynthesis (1 per cent.), both endothermic reactions. Thus, as a rule, only a very small proportion of the total energy available is utilised in photosynthesis, although with simpler aquatic plants such as *Chlorella*, where no transpiration occurs, Warburg found that up to 60 per cent. of the absorbed energy was used in photosynthesis. The higher plants can be divided roughly into two groups, depending on their reaction to high light intensity. The light-loving plants such as Larch, Birch, and, in general, natives of the prairie, show an increasing assimilation with increasing insolation. The shade-loving plants, on the other hand, such as Pine, and the undergrowth of forests, *e.g. Oxalis*, only show an increasing assimilation with light up to a comparatively low intensity. In most such plants, the chloroplasts, which are of various forms, lens-shaped, disk-shaped, plates, etc., can also protect themselves from too intense insolation; for in low light intensities they are turned with the greater diameter exposed to the light, while in high light intensities they are turned at right angles so that only the edge is exposed. Warburg and Negelein showed with *Chlorella* that both 'light' and 'shade' plants of the same species could be obtained simply by cultivating under different light intensities.

The effect of light intensity and temperature on photosynthesis must be considered together because of the following results. As would be anticipated in a **photochemical** reaction, with an abundant supply of carbon dioxide, the rate of assimilation is proportional to the light intensity, and is almost independent of the temperature between the normal values of 15° and 25° C. This, however, only holds for low light intensities. With stronger insolation, the light intensity may be doubled without altering the photosynthetic rate; that is, light is no longer the limiting factor, and an increase in the temperature from 15° to 25° C. doubles the rate of photosynthesis. Now a temperature increase of 10° which doubles the rate is characteristic of chemical and enzymatic reactions (van't Hoff's rule, p. 250), and hence it appears that assimilation involves a **chemical** as well as a photochemical reaction. These two reactions proceed simultaneously in the light; but because the photochemical one is dependent on light it is often called the '**light reaction,**' while the chemical reaction, because it is independent of light, is called the '**dark reaction.**'

Quality of the Light. The *chlorophyll spectrum* shows two regions of strong absorption, *viz.* in the blue-violet (wave-length about 470 μμ), and in the red region (700–650 μμ). Senebier and many later investigators have attempted to correlate maximum photosynthesis with the absorption spectrum of chlorophyll. The earlier workers found that maximum photosynthesis occurred in the red-yellow portion of the spectrum, but they did not pay sufficient attention to the fact that different parts of the spectrum have a different energy content. The true criterion should be the efficiency of the photosynthetic process for equal amounts of radiant energy at different wave-lengths. The most complete experiments so far have been those of Warburg and Negelein on *Chlorella*, using coloured screens to limit the spectrum. They found that the efficiency of the photosynthetic process decreased with decreasing wave-length; that is, that the red end of the spectrum was more efficient than the blue, with light of equal energy-value. Hence they concluded that there is no relation between the photosynthetic efficiency and the absorption spectrum of chlorophyll. One suggestion which has received support from more recent experiments (*e.g.* Gaffron's) is that the lower efficiency of the short waves is due to a partial absorption of this blue light by yellow colouring materials (*e.g.* the carotenoids) present in the chloroplasts. Hence it is probable that the efficiency is the same for all parts of the solar spectrum reaching the earth. The problem is complicated by the fact that the different wave-lengths have different effects on other metabolic activities of the plant, *e.g.* on the amount and nature of growth (p. 300), owing perhaps to a varying effect on the enzymes concerned, and to an alteration in the permeability. One more fact must, however, be noted; namely, that chlorophyll in the chloroplasts and in solutions fluoresces, and re-radiates incident yellow, green, or blue light partly as red light. In this way chlorophyll would increase the efficiency of the incident light for photosynthesis if the red end of the spectrum were the most efficient. This fluorescence is *excess energy*, and shows that the light energy absorbed is not all used up in the first few moments of illumination—presumably owing to the complexity of the system. From experiments with *Chlorella*, even when photosynthesis is at a maximum, the fluorescence is directly proportional to the light intensity. Hence all theories which assume that one chlorophyll molecule is required for each molecule of carbon dioxide combined are no longer tenable.

The Chemical Mechanism of Photosynthesis

The assimilation of carbon dioxide, the evolution of oxygen, and the formation of carbohydrates in the chloroplasts are the well-established facts of the photosynthetic process. It has also been shown that the number of volumes—and therefore (by Avogadro's hypothesis) the number of molecules—of carbon dioxide absorbed is equal to the number of volumes (molecules) of oxygen evolved. The **quotient of photosynthesis**, CO_2/O_2, is therefore unity (sometimes the inverse ratio is called the quotient).

Many theories as the mechanism of photosynthesis have therefore been proposed for this apparent photochemical reduction of carbon dioxide or carbonic acid to carbohydrates. The most popular was the intermediate formation of *formaldehyde*, which can, in dilute alkali, polymerise to sugars. Formaldehyde is, however, produced by the decomposition of many organic compounds, especially in light, so that its detection in irradiated plants does not necessarily mean that it is a product of photosynthesis. Again, formaldehyde in high concentrations is toxic to the plant, so that any theory as to its formation in the photosynthetic process must also postulate that it is immediately combined to give some inert compound, or is polymerised to carbohydrates. Various feeding experiments with formaldehyde appear to show that small amounts may be utilised by the plant in the synthesis of starch. But a great variety of substances can be fed to the plant with the formation of starch (*e.g.* the polyhydric alcohols, p. 25); thus the formaldehyde may merely upset the internal balance of the various processes in the leaf (including respiration, translocation, etc.), with the possible result of an increase in starch content.

Other theories were those of Willstätter and Stoll and of Maquenne, in which carbon dioxide and water added on to the chlorophyll molecule *via* the magnesium atom in the 'dark reaction'. The 'light reaction' then absorbed energy for an intramolecular rearrangement, with the evolution of oxygen, and the formation of either formaldehyde or a sugar-like polymer of it. The fluorescence experiments cited above disprove this theory.

Recent investigations, especially those utilising 'tracer' elements or isotopes, have separated the 'light' and the 'dark' reactions, and have made clear some of the mechanism of photosynthesis.

(*a*) Gaffron found that certain algæ could be adapted to hydrogen gas, and that they could *reduce carbon dioxide* in the *dark* without the liberation of any oxygen; in other words, **carbon dioxide reduction is part of the 'dark reaction'.** This absorption of carbon dioxide to form organic metabolites was known to take place in certain bacteria, *e.g.* the nitrifying bacteria, and the colourless sulphur bacteria, which obtain the required energy by the oxidation of simple substances. But in the coloured (green and purple) sulphur bacteria, which contain bacteriochlorophyll, a photosynthetic process takes place; that is, carbon dioxide is assimilated on irradiation, and instead of evolution of oxygen, oxidation of inorganic forms of sulphur takes place, *e.g.* hydrogen sulphide to sulphur, sulphur and sulphurous acid to sulphuric acid according to the strain of bacteria used. The general reaction is therefore:

$$CO_2 + 2H_2R \longrightarrow (CH_2O) + H_2O + 2R$$

In the higher plants, R is oxygen, and the equation becomes:

$$CO_2 + 2H_2O \longrightarrow (CH_2O) + H_2O + O_2$$

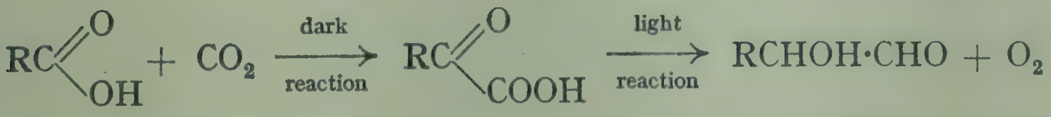
(*b*) Hill showed that isolated chloroplasts, which have no ability to reduce carbon dioxide, can produce oxygen by the photoreduction of ferric salts. Hence **oxygen evolution is part of the 'light reaction'.** This is called the Hill reaction, and indicates also the presence of an *enzyme* in the chloroplasts, either separate from, or part of, chloroplastin.

(*c*) Stauffer added reducible substances to suspensions of *Chlorella* in the absence of carbon dioxide, and found that in the light oxygen was evolved. Again, this shows that oxygen evolution is part of the 'light reaction', and also that this **oxygen comes** not **from carbon dioxide,** but **from the oxidation of water.**

(*d*) Ruben used carbon dioxide containing the radioactive carbon isotope C^{11} in the presence and absence of light. He found (i) that the carbon dioxide was assimilated in the dark; (ii) that this 'dark reaction' could take place in plants which contained no chlorophyll; (iii) that no radioactivity was present in chlorophyll after assimilation in the dark, and very little after assimilation during light exposure. Hence the assimilation of carbon dioxide occurs in the 'dark reaction'; this is the *primary reaction* of photosynthesis, and the chlorophyll molecule does *not* enter into this chemical reaction. Finally (iv) the products of the assimilation were in the water-soluble extract of the plant, but only a small percentage of the radioactive carbon was in sugars. In later experiments on *Chlorella*, the water-soluble extract resulting from the 'dark reaction' was treated with barium, and the bulk of the radioactive material was precipitated as a barium salt. Dry distillation of this yielded a radioactive carbon dioxide, and left a residue which contained only half of the original assimilated radioactivity. These results indicate that the reaction product consisted of **carboxylic acid,** so that the 'dark reaction' consists of the addition of carbon dioxide to some compound in the chloroplasts to form a carboxylic acid:

$$\mathrm{RH} + \mathrm{CO_2} \longrightarrow \mathrm{R{\cdot}COOH}$$

The 'light reaction' is the **reduction** of this carboxyl group with the liberation of oxygen. What the compound RH is has not yet been determined. Ruben suggested that it might be an aldehyde, in which case the reaction would be as follows:



$$\mathrm{RC}\begin{matrix}/\!\!/\mathrm{O}\\ \backslash\mathrm{OH}\end{matrix} + \mathrm{CO_2} \xrightarrow[\text{reaction}]{\text{dark}} \mathrm{RC}\begin{matrix}/\!\!/\mathrm{O}\\ \backslash\mathrm{COOH}\end{matrix} \xrightarrow[\text{reaction}]{\text{light}} \mathrm{RCHOH{\cdot}CHO} + \mathrm{O_2}$$

The addition of carbon dioxide to pyruvic acid ($\mathrm{CH_3{\cdot}CO{\cdot}COOH}$) occurs in the respiration process (p. 283) and simultaneous carboxylation and reduction occur in certain bacteria. Lipmann, using phosphorus (P^{32}) and carbon (C^{11}) isotopes, traced this latter reaction using acetyl phosphate as substrate.

$$CH_3{\cdot}COOPO_3H_2 + CO_2 + H_2 \rightleftharpoons CH_3{\cdot}CO{\cdot}COOH + H_3PO_4$$

He found that there was a rapid exchange of tracer phosphorus between the organic and inorganic forms, and of carbon between the carbon dioxide and the carboxyl group of the pyruvic acid. Emerson showed that in *Chlorella* the distribution of **phosphate** was different after irradiation both in the presence and absence of carbon dioxide. Phosphate in certain organic compounds has a high energy of transference (p. 258); this is of fundamental importance in respiration, and it is possible that in photosynthesis also, 'high energy' phosphate is the first stage in the photochemical or 'light reaction.' Then the reduction of the assimilated carbon dioxide would take place through a phosphate energy transference.

The Products of Photosynthesis

Sachs showed that **starch** was formed in the chloroplasts of leaves as a result of photosynthesis. Later investigators found that there were also sugars present, especially the hexoses **glucose** and **fructose** and the disaccharide **sucrose.** Only traces of pentoses, if any, occur free in leaves, although their condensation products, the pentosans, are among the components of cell-walls. Also maltose is probably absent from the living leaf, although it is rapidly formed from starch by the action of the enzyme diastase occurring in leaves and liberated in the process of drying and extraction.

It is relatively easy to explain the presence of the large variety of sugars which are found combined in the polysaccharides and as glycosides.

Several enolic forms of one hexose (*e.g.* glucose) can give rise to other hexoses on reverting to keto forms (p. 68), and inversion (exchange of positions) of the hydroxyl and hydrogen atoms attached to one of the carbon atoms may also occur by condensation with and subsequent removal of a molecule of phosphoric acid. This has been seen in the first stage of the fermentation process (p. 255). Again, the pentoses are derivable from the hexoses by decarboxylation of the uronic acids (p. 69).

Reserve carbohydrates in the higher plants are mostly sucrose and starch. The former functions in the plant as a temporary reserve, especially in the leaves, and also as a sugar for translocation. Starch, on the other hand, is found mostly in seeds, roots, and tubers, as a more permanent form of storage. Some plants, especially among the monocotyledons (p. 82), do not normally synthesise starch in the chloroplasts, although small amounts of this substance occur in the guard cells of the stomata. Most of these plants, however, can be induced to form starch under special conditions; *e.g.* by increasing the sugar content in the leaf by floating the leaves in sugar solutions (a 20 per cent. sucrose solution being required for Iris leaves), or by increasing the rate of photosynthesis by supplying excess carbon dioxide. Each plant has apparently its own concentration of sugars beyond which starch is formed, and this will vary with temperature. For instance, Smith found that the carbohydrate formed in Sunflower leaves was equivalent to the carbon dioxide assimilated. This carbohydrate was isolated as sucrose and starch, which appeared to be formed simultaneously. But at 10° C. more sucrose and less starch was synthesised in comparison with a higher proportion of starch and less sucrose at 20° C. This arrangement is an important factor in the mechanism whereby alpine plants resist the effects of cold; in such plants photosynthesis may still take place at low temperatures; but sugars are formed instead of starch, and the high concentration of sugars lowers the freezing-point of the cell-sap (p. 317).

Commencing with the work of Brown and Morris (1893), many investigations have been made of the carbohydrate content of green leaves, and of the fluctuations in the content of the various sugars, and of starch. In **'starch' leaves**—that is, leaves which build up starch normally—the starch increases in light and decreases in darkness. Starch is not the first carbohydrate formed in photosynthesis; in the leaf it is merely a temporary storage form of carbohydrate. Using detached leaves, so that there was no translocation and all the carbohydrates remained in the leaf, it was found that in the light *sucrose increased*, and the *hexoses* remained approximately *constant*. Brown and Morris found this for *Tropæolum* leaves; Davis, Daish, and Sawyer for Potato leaves; Miller for Maize leaves, to cite some examples. Also in **'sugar' leaves**—that is, leaves which do not normally store starch—sucrose increases in the light and the hexoses remain constant (Parkin on Snowdrop leaves). All these investigators concluded that **sucrose** was the first sugar of photosynthesis, and that the hexoses were formed from it. It was also shown that in the Snowdrop sucrose predominated in the tip, while hexose predominated in the lower part of the leaf. Again, in the Potato plant, the concentration of sucrose was greater than that of glucose in the leaves, but the reverse was found in the petioles. All this can be explained as follows: sucrose is the first sugar of photosynthesis, and therefore accumulates in the daytime; only sufficient of it is hydrolysed to keep up a constant content of hexoses; these are translocated to other parts of the plant and utilised in other syntheses and in respiration. Exactly the same data may, however, be explained in another way: a hexose is the first sugar of photosynthesis, but whenever it attains a certain concentration, sucrose is formed, and hence it is the content of sucrose, and not that of hexose, which fluctuates in the daytime.

Weevers brought forward experiments on variegated leaves of Ivy (*Hedera Helix*), of the Ash-leaved Maple (*Acer Negundo*), of the Hop (*Humulus Lupulus*), of *Pelargonium zonale*, and other plants to show that **glucose** was the first sugar of photosynthesis. He found in the green portion—that is, the only part where photosynthesis could take place—that both sucrose and hexoses were present, while in the non-green part only sucrose was present. He also showed that when such leaves were allowed to assimilate for a short time only, an increase in hexoses was found in the chloroplasts.

Various experiments have also been made to determine the *sugar* or sugars *of translocation*, especially in plants which store carbohydrates either as sugars or starch in underground organs. In most cases the migratory sugars appear to be glucose and fructose, and these are resynthesised to sucrose or to starch in the storage organ; *e.g.* in Sugar Beet, Mangold, *Canna edulis*, and Snowdrop.

Most leaves contain higher percentages of fructose than of glucose, but the hexoses, sucrose, and starch are all undoubtedly secondary products of photosynthesis. They are also interconvertible through the phosphorylase enzymes of respiration (p. 281), as well as through hydrolysing enzymes.

CHAPTER XXIV

RESPIRATION AND THE CARBON METABOLISM OF PLANTS

RESPIRATION is the mechanism whereby the plant receives energy for its vital processes of growth and movement and for the endothermic reactions involved in many of its synthetic activities. Respiration is often interpreted in a more restricted sense as the process in which oxygen is absorbed by the plant and carbon dioxide evolved; but the term should include all oxidative processes which release energy without necessarily producing carbon dioxide. There is in plants no elaborate mechanism for regulating the temperature of the tissues as in animals, hence when more energy is liberated in respiration than is used up in the metabolic changes, the excess appears as heat. This is most evident in closed flowers, in which the temperature may rise appreciably above that of the outside air.

The Respiration Rate

The respiration rate differs in different types of plants, and does not remain constant throughout the life of one plant. The highest rate is found in rapidly metabolising organs such as buds, and there are usually *two maxima* for higher plants, one at germination and the other at the opening of the flower-buds. Respiration then slows down with the ageing of the tissue or of the whole plant.

The respiration rate also depends on many internal and external factors, including the amount of *water* and *oxygen* available to the plant, the *temperature*, and the *light*. Light, in addition to its function in photosynthesis and therefore its effect on the amount of respirable material available, may affect respiration through a change in the permeability of the cell. Temperature affects respiration, as it does all chemical reactions, in accordance with van 't Hoff's law, within the limited temperature range in which protoplasmic activity is possible, but in addition it may affect the extent of oxidation of the respirable material, especially in the case of succulents (*vide infra*). Here also the effect of oxygen supply may be a determining factor. It must be remembered that—unlike the carbon dioxide necessary for photosynthesis—oxygen intake by the plant is not entirely dependent on open stomata.

The respiration of detached plant tissues, especially ripe fruits, is a separate phenomenon, which is most important commercially for their transport and storage. After maturity in fruit comes the stage termed 'senescence,' characterised by a rapid increase to a maximum in the respiratory rate (p. 311), which then gradually declines to zero and causes the 'death' of the fruit, all the material available for respiration having been used up.

The influence of *anæsthetics* on respiration is now being used in the artificial ripening of fruits (*e.g.* the use of ethylene), and in breaking the rest-period of bulbs, tubers, etc. (*e.g.* the use of ether, chloroform, etc.). It is not yet clear to what this effect is due; suggestions are that these anæsthetics increase the permeability of the protoplasm to oxygen and so increase the respiratory rate, or that they affect some oxidising enzyme-substrate mechanism.

Respiratory Quotient

The normal substrate for respiration in plants is undoubtedly carbohydrate, probably in the form of hexose. The complete oxidation of a hexose is shown by the equation:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674 \text{ Cals.}$$

This amount of energy, measured in heat units (Calories), is *independent* of the number and nature of the intermediate steps, and therefore will be the same so long as carbon dioxide and water are the ultimate products. This equation also gives a value *unity* for the ratio CO_2/O_2, which is called the respiratory quotient. Actual measurement of this ratio shows, however, that it can diverge from unity quite markedly, both in different plants and at different stages in the life of the same plant. For instance, in the *ripening of fatty seeds*, which involves a change from carbohydrate to fat (containing less oxygen in the molecule), less oxygen is absorbed, and hence the respiratory quotient is *greater* than unity. During the *germination* of such seeds, on the other hand, there is a large absorption of oxygen and the respiratory quotient falls below unity (p. 280).

Again, if the oxidation of carbohydrate is not as complete as is shown by the above equation, but stops with the formation of intermediate products (chief among which are the plant acids such as malic and oxalic acids), then there will be less carbon dioxide formed, and therefore the respiratory quotient will be less than unity. Now acids accumulate at night in **succulents,** *e.g.* in the *Cactaceæ*, *Crassulaceæ*, and *Mesembryanthemum* (p. 122), and there is a concomitant fall below unity in the respiratory quotients of these plants, *e.g.* for *Phyllocactus* in darkness, $Q = 0{\cdot}33$, for *Opuntia*, $Q = 0{\cdot}03$. Both a restricted oxygen supply and low temperatures are held responsible for a partial oxidation of the carbohydrate, as the acids disappear in the plant at higher temperatures, even in darkness. According to this theory, the oxygen supply is low at night, since no oxygen is produced intracellularly by photosynthesis, and because there may be a limited gas exchange due to the special structure of succulents—some of these having a relatively impermeable cuticle, while in others, *e.g.* Cacti, the stomata are closed during most of the day. That the explanation is probably not quite so simple has already been mentioned (p. 122).

Oxalic acid, like the other acids, can be further oxidised; but in most cases it is removed from participating in the active metabolism of the plant by precipitation as the calcium salt in the cells of stems and of wood (p. 115). Cross and Bevan found that large quantities of calcium oxalate produced in respiration are deposited in the bark of a Himalayan tree, *Shorea robusta*. It must also be remembered that in some plants these acids are not products of respiration of carbohydrates, but of the deamination of amino-acids (p. 294). Again, ripening fruit contains both acid and carbohydrate; during ripening the acid disappears, probably being used up as a respiration substrate. Ripening grapes contain both hexoses and tartaric acid—both possible substrates for respiration—and it has been found that at a temperature below 15° C., $Q = 1$, showing that only hexoses are being oxidised, whereas above 15° C., $Q > 1$, owing to the oxidation of tartaric acid as well.

The Substrate for Respiration

Carbohydrate is the normal substrate for respiration, and it occurs in the plant in three main forms, *viz.* the hexoses (glucose and fructose), sucrose, and polysaccharides (especially starch). These are all interconvertible through the hexose phosphates (p. 255) by the action of the phosphorylases. Also in tissues in which photosynthesis is taking place, the first product of photosynthesis is a possible substrate for respiration. Many measurements have been made on the variation in content of the stable forms of carbohydrate, in attempts to correlate one of these with a preferential substrate for respiration. Spoehr finds in *Cactaceæ* that with a low water supply and high temperature the hexoses are respired, while with ample water supply and low temperature the polysaccharides are oxidised first. Most of the work of this kind has been done on the storage of ripe fruit; here there is hydrolysis of sucrose to hexoses, and a loss of total sugars due to respiration. Onslow has shown from the analytical data of several workers on the constituents of pears, bananas, etc., that during part of the storage life (but see p. 313) γ-*fructose* may be the preferential substrate for respiration, because (i) approximately half the loss in sucrose is equal to the loss of total sugars, and (ii) the gain in hexose content is equal to half the loss of sucrose, being due to the glucose part of sucrose. However, glucose can in some cases be used as a respiration substrate when the sucrose reserves have been depleted, and to what extent these results on detached tissues apply to the whole growing plant has not been fully investigated. Fat is in some cases also a substrate for respiration, *e.g.* in the germination of fatty seeds; it is first converted into carbohydrate, as is shown by the concomitant decrease of fat content and increase in soluble carbohydrates. The first reaction will be the addition of oxygen to the unsaturated acids, and hence there will be a large absorption of oxygen, which explains why in the germination of fatty seeds, Q is less than unity.

The Mechanism of Respiration

Respiration in the higher plants is very closely related to **fermentation**; indeed, the first stage in respiration, which includes the formation of some carbon dioxide but not the intake of oxygen, appears to be identical with part of the fermentation process. If respiration is allowed to proceed in an inert atmosphere, *e.g.* of hydrogen or nitrogen, ethyl alcohol and carbon dioxide are actually produced; this is termed anaerobic respiration. If respiration proceeds in air, the **first stage** corresponds to *fermentation* as far as the production of pyruvic acid (end of Stage II, p. 256); and the **final stage** is the *oxidation of the intermediate products from the fermentation process*, with the formation of carbon dioxide and water. The whole process of fermentation does, however, occur in over-ripe fruits, alcohol being formed.

The evidence for the relationship between the respiration of higher plants and Stages I and II of fermentation may be summarised as follows:

(1) A **zymase**-like enzyme occurs in many of the higher plants, *e.g.* leaves and roots of Beet, Potato tubers, seedlings of Pea, Lupin, and Barley, and most germinated seeds. If the enzyme complex is isolated by precipitation from the plant juice with alcohol, it will ferment sugars to alcohol and carbon dioxide. The presence of **cozymase** has also been shown in Peas, Lupin, and ripening Bananas.

(2) A **hexose-phosphatase** has been shown to occur in some

plants, *e.g. Nicotiana tabacum*, and in experiments on the respiration of germinated Peas, twice as much carbon dioxide was evolved when phosphate was added.

(3) Where the substrate is starch instead of hexose, as in starch-storing seeds and tubers, two enzymes convert the **starch** *via* **glucose-1-phosphate** (Cori's ester) to the **glucose-6-phosphate** (Robinson's ester of fermentation, p. 255). Similar enzymes in the animal system convert storage glycogen to the same ester. Hanes isolated a *phosphorylase* from many plants, especially from Potato and from Pea seeds, capable of converting any 1 : 4-glucopyranose polymer (starch or glycogen) to glucose-1-phosphate. It also appears to be able to synthesise disaccharides (*e.g.* sucrose from glucose-1-phosphate and fructose). The second enzyme, *phosphoglucomutase*, present in both animals and plants, converts the glucose-1-phosphate to glucose-6-phosphate.

(4) The conversion of carbohydrate to **pyruvic acid** in plant respiration was shown by James for Barley. He showed (i) that in the cell-sap pyruvic acid was formed from glucose in the presence of adenylic acid, and (ii) that 1 : 3-*diphosphoglycerate* was formed from fructose-1 : 6-diphosphate (p. 256).

Stage III of fermentation has also a parallel in plant aerobic respiration:

(*a*) **Carboxylase** is well distributed in plants. It has been isolated from Peas, Beans, Lupin seeds, Wheat grain, Potato tubers, Beet, Bananas, etc., and the extract will produce carbon dioxide and acetaldehyde from pyruvic acid. **Cocarboxylase** (p. 257) has also been isolated from various plant tissues. Separation of the enzyme in the Jack Bean shows that both cocarboxylase and *magnesium* are necessary for carboxylase activity.

(*b*) The presence of **acetaldehyde** has been detected during the respiration (sometimes anaerobic) of Peas, Beans, cereal grain, and Poplar catkins. It is also produced in Apples stored under anaerobic conditions, either through absence of oxygen or presence of too high a concentration of carbon dioxide. Using the 'dimedone' condensation product (p. 59), acetaldehyde has also been detected under aerobic conditions in flowers, leaves, and seedlings. The acetaldehyde so formed is then oxidised by atmospheric oxygen to carbon dioxide and water.

The pyruvic acid from Stage II is not, however, all decarboxylated as in Stage III. It is also converted by a series of enzymatic transformations called the **carboxylic acid cycle** into the **plant acids.** These in turn can be oxidised by atmospheric oxygen to the ultimate products of respiration, *viz.* carbon dioxide and water, and in addition, some of them are undoubtedly the primary *source* of *amino-acids* for **protein synthesis.** Again, pyruvic acid may be oxidised directly, especially in presence of phosphate. Lipmann showed that in the presence of inorganic phosphate pyruvic acid was oxidised to **acetyl phosphate** by the *Bacterium Delbrueckii :*

$$CH_3{\cdot}CO{\cdot}COOH + O_2 + H_3PO_4 \longrightarrow CH_3{\cdot}COOPO_3H_2 + CO_2 + H_2O_2$$

Acetyl phosphate can also arise by the *dehydrogenation* of pyruvic acid, in the presence of phosphate and of a hydrogen acceptor, with evolution of carbon dioxide:

$$CH_3{\cdot}CO{\cdot}COOH + H_3PO_4 \xrightarrow{-2H} CH_3{\cdot}COOPO_3H_2 + CO_2$$

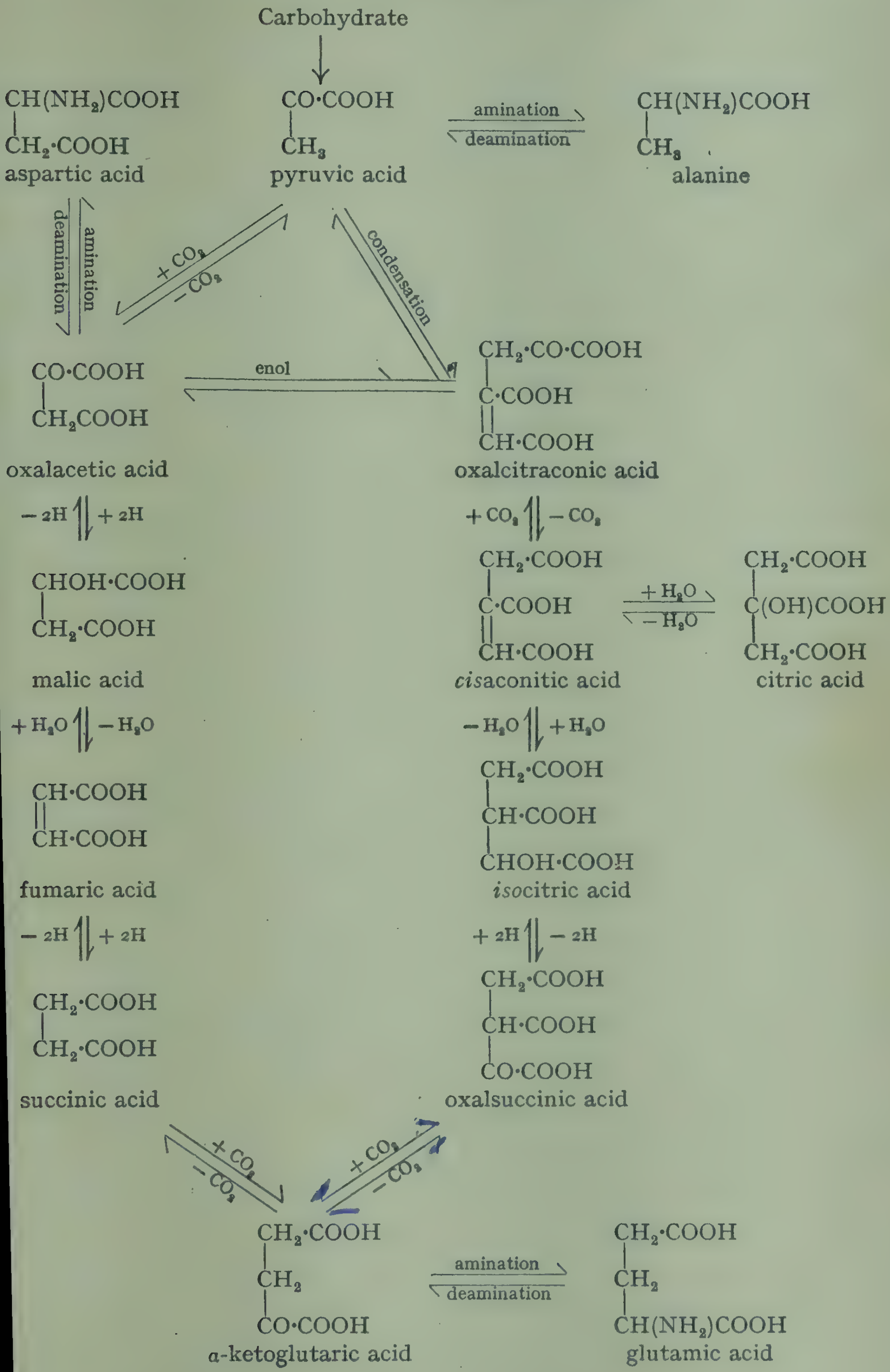
If this phosphate is an *organic* phosphate donor such as adenosine triphosphate (p. 170), then the acetyl phosphate so formed will have the *high energy* content of the ~ph group (p. 259). This compound may then react, depending on the enzyme systems present, to give either acetyl and ~ph, or acetyl~ and inorganic phosphate. Here we have an energy-rich two-carbon unit, the most likely building unit in **fat synthesis.**

The Carboxylic Acid Cycle (Modified Krebs Cycle)

Various carboxylic acids occur in traces in animal systems, and with them enzymes which bring about their interconversion. Krebs showed how these transformations were related, and his original 'cycle' has been modified to conform to more recent results, especially those obtained by the use of 'tracer' elements. (Other names for this cycle are *citric acid cycle* and *tricarboxylic acid cycle*). These same acids occur in plants, some of them in high concentrations, and there is no doubt that the carbon metabolism of higher plants includes at least part of this same cycle. In addition, the primary **synthesis of amino-acids** is possible by the *amination* (p. 135) of certain of these acids.

That carbon dioxide plays a fundamental role in cellular respiration in the carboxylation of pyruvic acid to oxalacetic acid has been shown for animals, bacteria, yeast, algæ, and higher plants (ground plant roots were employed). The carbon of the carbon dioxide or of bicarbonate supplied to the tissues was tagged by using *isotopic* forms; the stable isotope C^{13} and the radioactive forms C^{11} and C^{14} were all employed. Adenosine triphosphate (ATP) appears necessary for this carboxylation. Carbon isotopes

The Carboxylic Acid (Krebs) Cycle



were also used in the animal system to show the formation of citric acid as a side-product.

Experiments on detached Tobacco leaves have shown the existence of the carboxylic acid cycle, and several of the acids were isolated. Malic and citric acids occur in relatively high proportions in many of the higher plants, and in small amounts they are probably universally present. Succinic, fumaric, and *iso*citric acids have also been isolated from various plants. Oxido-reductases or **dehydrogenases** catalysing several of these reactions are also present, and dehydrogenases which act on citric acid, *iso*citric acid, malic acid, succinic acid, and glutamic acid have all been isolated. The cozymase of yeast can act as a coenzyme for some of these reactions, *e.g.* for the malic acid dehydrogenase. Many seeds (*e.g.* Wheat, Rye, Peas, Soya Beans) contain an enzyme *aconitase* which catalyses the reaction *cis*aconitic acid to *iso*citric acid.

*Iso***citric acid** is the most oxidisable substrate in the cycle, and experiments indicate that **final oxidation to carbon dioxide and water** occurs at this point. *Transaminations* are also possible within the cycle; *e.g.* between glutamic acid and oxalacetic acid to give α-ketoglutaric acid and aspartic acid.

The Activation of Oxygen and Substrate

Atmospheric oxygen has no effect on fermentation; therefore in aerobic respiration there must be **activation** of either the **oxygen,** or the **substrate,** or both, so that the final oxidation of the substrate to carbon dioxide and water is accomplished. This terminal oxidation probably occurs not at one but at several stages in the breakdown (glycolysis) of carbohydrate, summarised in the schemes for fermentation and the carboxylic acid cycle. For such oxidations a number of enzyme systems of *hydrogen transfer* have been isolated and studied. The phosphopyridine nucleotide coenzymes are known to transfer hydrogen to the riboflavin coenzymes, these in turn transfer hydrogen to some of the cytochromes, and the latter react directly with atmospheric oxygen. On the other hand, James showed that in the respiration of Barley, the oxidation of carbohydrate took place *via* triose phosphate through cozymase (coenzyme I), dehydrogenase, ascorbic acid-ascorbic acid oxidase, and finally atmospheric oxygen.

There follows a brief summary of the more important modes of terminal oxidation, but the complete scheme of the interdependence of all the enzymes is not yet known.

The Cytochromes. The cytochromes are thermostable respiratory pigments, first isolated from yeast by Keilin, and since found to be widely distributed in animal tissues, in bacteria, and in the higher plants. In the reduced form, cytochrome has an absorption spectrum with several distinct bands, and further analysis shows that the cytochrome consists of a mixture of substances with one absorption band in common, and each of the other bands belonging to a separate cytochrome. Each cytochrome is capable of independent oxidation and reduction, some requiring a cytochrome oxidase (p. 263). On passing air through an aqueous suspension of yeast, the cytochromes are oxidised, and instead of definite absorption bands, only a faint general absorption is obtained, while on stoppage of the air current the bands of the reduced cytochromes reappear. Indophenol oxidase of yeast was shown by Keilin to be identical with Warburg's 'respiratory enzyme', part of the cytochrome system (*loc. cit.*). Oxidised cytochromes can be reduced either by the living cell, or *in vitro* by various organic compounds acting as hydrogen donors, *e.g.* the sodium salts of pyruvic and succinic acids, which *in vivo* are activated by dehydrogenases. Chemically, the cytochromes are iron-porphyrin compounds or hæmes with their specific activating proteins. Some (a_1 and a_3) are **direct oxidases,** activating atmospheric oxygen to make it an electron (hydrogen) acceptor, others (b_2 and c) are electron transporters between intermediate portions of the oxidation-reduction system, with the simultaneous reversible oscillation of the valency of the iron between ferrous and ferric.

Ascorbic Acid. Ascorbic acid is another thermostable catalyst; it was first investigated in connection with respiration, when it was called **hexuronic acid,** but it has since been identified with vitamin C (p. 70). Szent-Györgyi isolated it as a crystalline substance from animal tissues, but found it also widely distributed in the higher plants, *e.g.* in fruits of Orange and Lemon, in leaves of Cabbage, and roots of Radish and Turnip. Ascorbic acid has strong reducing properties, and undergoes two kinds of oxidation, one reversible, the other irreversible. In the former type, which takes place in the living cell, ascorbic acid functions as *hydrogen donor* to substrates in the cell. Various enzyme systems coordinate with ascorbic acid. A specific **ascorbic acid oxidase** has been found in many plants, *e.g.* Cabbage, Carrots, Spinach, and Apples, and has been shown to increase during the active stages of germination in Oats, Rice, Barley, Peas, and Tomatoes, and in sprouting Potatoes. This ascorbic acid—ascorbic acid oxidase system is able to catalyse the oxidation of lactic acid to pyruvic acid in Barley sap. However, it is more likely that dihydroxyphenylalanine and tyrosinase (p. 262) form the universally distributed activating enzyme system for ascorbic acid.

Glutathione. Glutathione, a tripeptide (p. 157), is one of the most important thermostable catalysts of animal cells. A substance with similar reactions has been detected in plants, *e.g.* in seeds of the Pea, while glutathione itself has been isolated from sprouting tubers of Potato. Glutathione (G—SH) can reduce dehydroascorbic acid in the presence of a dehydrogenase, as follows:

$$\text{Ascorbic acid} + \tfrac{1}{2}O_2 \xrightarrow{\text{dehydrogenase}} \text{dehydroascorbic acid} + H_2O$$

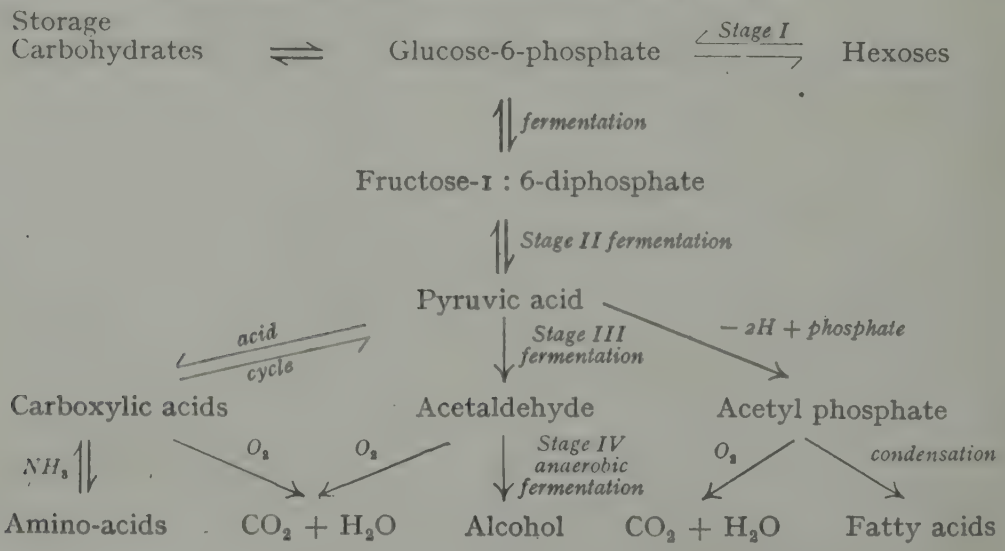
$$\text{Dehydroascorbic acid} + 2\text{G—SH} \longrightarrow \text{ascorbic acid} + \text{GS—SG}$$

Glutathione is also an activator of sulphydryl groups, and as these are present in, and necessary for, the activity of many of the activating proteins (apoenzymes) of the fermentation process, it may play an important part in carbohydrate metabolism. Pure crystalline glutathione oxidases fat only, but in the plant its action is bound up with that of enzyme systems. In addition to its effect on the enzymes mentioned above, it also activates the proteolytic enzymes, probably again because of the sulphydryl grouping (p. 159).

Other enzyme systems, some of which play a part in the fermentation and carboxylic acid cycle stages of respiration, probably also take part in the final oxidation. These would include the flavoproteins (the source of Palladin's pigment theory), and hæmoproteins other than the cytochromes.

Summary of Carbon Metabolism

The respiratory process and the production of metabolic units for synthetic processes may be summarised as follows:



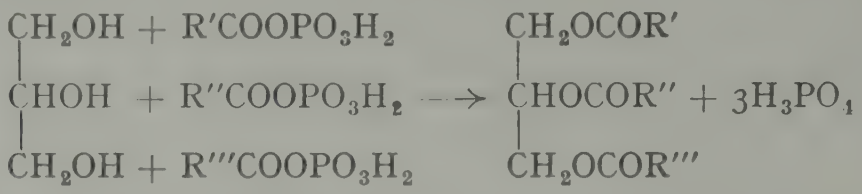
Blackman found that in aerobic respiration there is about three times as much carbohydrate decomposed as is finally oxidised to carbon dioxide and water, and also that there is more carbon dioxide evolved in anaerobic respiration (using an atmosphere of nitrogen) than in aerobic respiration. Hence some of the products of the breakdown of carbohydrate (glycolysis) must take part in synthetic processes, especially in the presence of oxygen.

Respiration of all living tissue is now recognised as a series of oxidation-reduction reactions, most of them reversible, which transfer electrons in a series of graded steps from the oxidisable substrate to molecular oxygen. These reactions are controlled by enzymes which are conjugated proteins; in the protein or apoenzyme lies the specificity of the enzyme, while the coenzymes are the molecules which transfer electrons in the oxidation-reduction reactions, and in other cases transfer phosphate, methyl groups, amino groups, etc. In all such reactions there are energy changes, and it has been shown that many of the coenzymes are molecules with a high energy of transfer of electrons, phosphate groups, methyl groups, as the case may be. This has been most studied in the transfer of phosphate (p. 255), where in the fermentation system phosphate is introduced as an inorganic ion, and then is shuttled by the adenylic group of coenzymes from one substrate to another, and finally removed to regenerate the starting coenzymes. For example, thiamine can act as a phosphate acceptor from adenosine triphosphate (in yeast), and is thus converted to the diphosphothiamine of cocarboxylase:

$$2\mathrm{ATP} + \mathrm{Th} \rightleftharpoons 2\mathrm{ADP} + \mathrm{DPTh}$$

Fat Synthesis. It has been shown (p. 47) that the fats are formed from carbohydrates, and it seems most probable that the fatty acids and glycerol are synthesised separately. A mechanism for the synthesis of glycerol as a side-reaction in the fermentation process has been shown (p. 258), and acetyl phosphate and pyruvic acid are formed from carbohydrate in respiration. These considerations lead to the most widely accepted explanation of the mechanism of fat synthesis.

Acetyl phosphate, formed from hexose *via* pyruvic acid in respiration (p. 282) contains a high energy phosphate bond, and provides a *two-carbon unit* that accounts for the fact that the naturally-occurring fatty acids all contain an even number of carbon atoms. These acetyl groups probably condense to form acyl phosphates, which can condense with glycerol to form the fat, as follows:



$$\begin{array}{l} CH_2OH + R'COOPO_3H_2 \\ | \\ CHOH + R''COOPO_3H_2 \\ | \\ CH_2OH + R'''COOPO_3H_2 \end{array} \longrightarrow \begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR'' \\ | \\ CH_2OCOR''' \end{array} + 3H_3PO_4$$

This condensation of acetyl phosphate does not, however, explain why such high percentages of oleic, linoleic, and palmitic acids are found in all plants.

A somewhat different suggestion is that **acetaldehyde** and **pyruvic acid,** both intermediate products of respiration, undergo aldol condensation (p. 58), followed by decarboxylation, and the new aldehyde then takes part in further aldol condensations. Again, this will always lead to acids containing an even number of carbon atoms:

$$\underset{\text{acetaldehyde}}{CH_3{\cdot}CHO} + \underset{\text{pyruvic acid}}{CH_3{\cdot}CO{\cdot}COOH} \longrightarrow CH_3{\cdot}CHOH{\cdot}CH_2{\cdot}CO{\cdot}COOH \xrightarrow{-H_2O}$$

$$CH_3{\cdot}CH{=}CH{\cdot}CO{\cdot}COOH \xrightarrow{-CO_2} CH_3{\cdot}CH{=}CH{\cdot}CHO$$

CHAPTER XXV

NITROGEN METABOLISM

Theoretical Considerations

In addition to effecting the photosynthesis of carbon compounds from carbon dioxide and water, the living plant absorbs inorganic nitrogenous compounds from the soil by means of its roots, and builds up the **proteins**; these complex nitrogenous substances form the protoplasm and also serve as storage materials. In normal soils the inorganic salts concerned are usually **nitrates**, even when the fertilisers applied are ammonium salts, for the latter are 'nitrified' *via* nitrites to nitrates by the soil micro-organisms (*Nitrosomonas* and *Nitrobacter*). Evidence is, however, accumulating that some plants, especially the grasses, can absorb ammonium salts from the soil; many plants, moreover, can utilise ammonia from culture solution. The relative ease of assimilation of ammonium salts and nitrates depends partly on the age of the plant—ammonia absorption predominating in the younger stages of plant growth, and that of nitrate later—and partly on the reaction of the nutrient medium. The use of 'tracer' nitrogen of atomic weight 15 has shown that growth is proportional to protein synthesis. In experiments on monocotyledon leaves, young leaves showed a high intake of nitrogen, and a corresponding low carbohydrate/nitrogen ratio, because of the rapid synthesis of new protoplasm. With increasing age of the leaves, the ratio correspondingly rises, and reaches a maximum at maturity.

In the plant, nitrate is assumed to be successively reduced to nitrite and then to ammonia. Enzymes capable of reducing nitrates to nitrites are known (p. 264), and maximum reducase activity in the Apple is concomitant with bud swelling and the consequent synthesis of new tissue in the early spring. The reduction of nitrite to ammonia is less well authenticated except in the experiments of Eckerson. She found that if Tomato plants, containing much carbohydrate but little nitrogen, were fed with calcium nitrate solution, nitrite could be detected in all tissues after twenty-four hours, and in thirty-six hours ammonia was accumulating and nitrite decreasing. Sometimes the nitrate travels as such to the leaves; in other plants, *e.g.* Asparagus, Polyanthus, and Narcissus, nitrate disappears, and ammonia and even organic nitrogenous compounds appear in the roots. There is a possibility that instead of ammonia, *hydroxylamine* (NH_2OH) is the active form of nitrogen produced from the assimilated nitrate. It is for instance produced in the fixation of nitrogen by the symbiotic bacteria in the root nodules of the *Leguminosæ*.

Considering the difficulties which arise in an attempt to formulate the mode of synthesis of the relatively simple carbohydrates, it is not surprising that an elucidation of all the steps in the building up of the proteins has not been achieved; for the proteins (p. 130) are complex compounds structurally composed of amino-acid units, which themselves belong to a variety of aliphatic, cyclic, and heterocyclic types. Amino-acids are also found in plants and the most probable mechanism for the *synthesis of proteins* is by the **condensation of preformed amino-acids.** This, however, presupposes that at every growing point where protein is being synthesised, all of the necessary amino-acids are present, and these must also have been built up separately by the plant. It has been definitely established that the **primary nitrogenous compounds synthesised** are **aspartic acid** and **glutamic acid.** When nitrogen of atomic weight 15 is fed to rapidly growing plants in the form of ammonium chloride ($N^{15}H_4Cl$), the 'tracer' nitrogen is rapidly assimilated and appears in the amides, the amino-acids, and the proteins, but the highest concentration was found in aspartic acid and glutamic acid. These are formed *de novo* by the *amination of keto-acids* produced in respiration, and they are the primary amino-acids supplying the nitrogen for the synthesis of the other protein constituents. The first reaction in protein synthesis is therefore **amination,** and the second is **transamination,** in which the amino group is transferred from one compound to another.

Transamination. The carboxylic acid cycle (p. 283) shows the formation of aspartic acid and glutamic acids; aspartic acid can also arise directly from fumaric acid by addition of ammonia. The following three reactions can take place:

(1) glutamic acid + oxalacetic acid $\rightleftharpoons$ α-ketoglutaric acid + aspartic acid
(2) glutamic acid + pyruvic acid $\rightleftharpoons$ α-ketoglutaric acid + alanine
(3) aspartic acid + pyruvic acid $\rightleftharpoons$ oxalacetic acid + alanine

The enzyme controlling transamination is called an **aminopherase** or a **transaminase.** Transaminase activity has been demonstrated in the *Leguminosæ* (Pea, Lupin, and Clover) and in the *Gramineæ* (Oat and Barley). It is possible that there is only one enzyme, transaminase. *Pyridoxal phosphate* (p. 171) appears to be the prosthetic group, and equation (1) above can therefore be written in two stages, the enzyme being regenerated after it has transferred an amino group:

(1*a*) glutamic acid + pyridoxal $\rightleftharpoons$ α-ketoglutaric acid + pyridoxamine

(1*b*) oxalacetic acid + pyridoxamine $\rightleftharpoons$ aspartic acid + pyridoxal

The **amides, asparagine** and **glutamine** (p. 138), are formed from these 'primary' amino-acids, and they may themselves enter into similar transamination reactions. In many instances, however, they act as a *reserve*—in a soluble and comparatively stable form—of their respective acids, translocating them to active points of protein synthesis.

Synthesis from Amino-acids. How all the other amino-acids are built up is not yet understood. The simpler types are derivable from aspartic and glutamic acids by reactions which include transamination, oxidation, hydrolysis, etc., which occur in all plants, but the formation of the aromatic and heterocyclic amino-acids is still unexplained, and it is difficult to see how this could be done without postulating a different mechanism for each compound. If the proteins are synthesised from the component amino-acids, then proteolytic enzymes will play a part. They have been shown to catalyse *in vitro* the synthesis of polypeptides. Another possibility is that reactions take place on the side-chains of proteins, *e.g.* transamination on an amino group, which would change one protein into another without the necessity for complete hydrolysis to the constituent amino-acids. One instance is the possibility of tyrosinase acting on the tyrosine part of a protein molecule, and other enzymes can activate sulphydryl groups.

Synthesis from Simple Molecules. Since phosphate bonds are the energy carriers in carbohydrate metabolism, it is not unlikely that they also play a part in protein metabolism. It has been suggested that, corresponding to acetyl phosphate, the building unit of the fats, **aminoacyl phosphates** (amino-acetyl phosphate and higher homologues) may condense with the amino groups of phosphorylated amino-acids to form the polypeptide chains of the proteins:

$$\mathrm{R(NH_2)COOPO_3H_2 + R'(NH_2)COOPO_3H_2 + R''(NH_2)COOPO_3H_2}$$
$$\mathrm{+\ etc.} \longrightarrow \mathrm{R(NH_2)CONHR'CONHR''CO - - - - \quad + xH_3PO_4}$$

In support of this thesis, it has been shown that both respiration and protein synthesis in Potato discs are increased by an increased supply of phosphate in a manner which implied a specific response of protein synthesis to phosphate.

Experimental Results

To locate protein synthesis in tissues and to show which products accumulate and which appear to be translocated necessitates analysis for protein, for amino-acids, for amide nitrogen (sometimes for asparagine itself), and for ammonia. The latter, however, is toxic in high concentrations, and therefore can never occur in more than very small amounts unless as the ammonium salts of acids. There are several points of attack in examining the plant for nitrogenous compounds, namely, the green leaf, the germinating seed, and the ripening seed; also in artificial 'feeding' experiments, the results depend in each instance upon whether nitrogen is in excess or lacking, and whether there is an abundance or a scarcity of carbohydrate. In later experiments, the nitrogen isotope N^{15} was used in the form of its ammonium salt, $N^{15}\,H_4Cl$; the nitrogenous compounds in the plant were then examined for their percentage of the isotope, and in this way the path of nitrogen metabolism in the plant could be traced. The investigators who have contributed most to the problem of protein synthesis include Schultze, Prianischnikov, Mothes, Chibnall, and Vickery.

The Green Leaf. The main seat of protein synthesis is in the green leaf, and it has been shown that *protein accumulates in the daytime*, especially in young leaves, *and decreases in the dark.* That the synthesis of protein is **independent of light** has been proved by several investigators, but it *is* dependent on a supply of carbohydrate, normally furnished in the daytime by the photosynthetic process. In *detached leaves* in the dark, protein hydrolysis takes place and amino-acids are formed, but if the leaf contains some carbohydrate, then **amides** gradually accumulate. If, however, the leaf is treated with a narcotic, *e.g.* chloroform, which stops all synthetic reactions, ammonia accumulates. Hence the amino-acids must break down to ammonia, and it has been shown that this reaction requires the presence of oxygen; and then, with a supply of carbohydrate, amides—especially asparagine—accumulate. These changes may be summarised as follows:—

$$\begin{tikzcd}
\text{Protein} \arrow[r, "\text{hydrolysis}"] & \text{amino-acids} \arrow[r, "\text{oxygen}", "\text{present}"'] & \text{ammonia} \arrow[r, "\text{carbohydrate}", "\text{present}"'] \arrow[d, "\text{narcotise}"] & \text{amides} \\
 & & \begin{array}{c}\text{ammonia}\\ \text{accumulates}\end{array} &
\end{tikzcd}$$

There are therefore two reactions taking place in the leaf: (*a*) the primary synthesis of protein, (*b*) the hydrolysis of protein. In young leaves, in presence of plenty of carbohydrate, synthesis predominates. In old leaves, even with a supply of carbohydrate, the supply of protein is not maintained, hydrolysis predominating over synthesis. There is in both cases in the dark, and in old leaves even in the daytime, a transference of protein by means of its hydrolysis products to the growing points of the plant, where protein is resynthesised in the meristematic tissues. This synthesis of protein in young leaves at the expense of that in older leaves is hastened by a low water supply. Thomas, working on the nitrogen metabolism of Apple trees, has shown that during rapid growth the protein synthesised by the young leaves migrates in the form of soluble amino-acids and amides to the shoots, where some is used in building the new tissue and some is stored in the phloem. This last reserve material is then mobilised rapidly, with the synthesis of new protein, during bud formation. In the autumn, hydrolysis predominates over synthesis in the old leaves; therefore a migration of nitrogenous products takes place, and these are stored mainly in the first and second year shoots. There is, in fact, an accumulation of nitrogen both in the wood and bark of trees in the dormant stage, and this diminishes rapidly during the growing period. Later experiments by Maskell and Mason show that nitrate moves upwards through the *wood*, while organic nitrogenous compounds show a decreasing concentration from the leaves through the petioles to the *bark*. Hence protein synthesis from inorganic nitrogen takes place in the leaves; moreover, from the leaves, hydrolysis products of protein move through the bark to other parts of the plant.

If young detached leaves are kept in the light and supplied with ammonia or with asparagine, synthesis of protein takes place. If the same leaves are kept in the dark, but supplied with carbohydrate (either glucose or sucrose solution) and with ammonia or asparagine, again an increase of protein is obtained. Hence *in young leaves in the presence of carbohydrate* (either photosynthesised or supplied artificially) *protein can be synthesised both from ammonia and from asparagine.* If young detached leaves containing only a little carbohydrate are kept in the dark and supplied with ammonia, amides accumulate. If they contain no carbohydrate and are kept in the dark, then amides are formed by hydrolysis of the protein already in the leaf, and supplying the leaves with ammonia has no effect other than that it may cause ammonia poisoning of the tissue. If asparagine is supplied instead of ammonia, some asparagine is assimilated and stored unchanged. From these experiments it would appear that the amides are not only formed in the hydrolysis of protein, but that they also serve in the synthesis of protein.

In fact, they are **reserve forms of soluble nitrogen.** In the Potato plant, for instance, feeding with ammonia gave an increase in amides, especially asparagine, in the growing shoots, and on cessation of the nitrogen supply, the asparagine reserves were utilised as a source of nitrogen for protein synthesis. In the Beet, the storage amide was glutamine. Experiments in which Tobacco plants were fed with ammonium chloride containing isotopic nitrogen showed that aspartic and glutamic acids contained the highest concentration of the isotope. Björksten injected nitrogen-starved Wheat seedlings with nutrient solutions containing ammonia, and glucose or pyruvic acid. He found that the protein was synthesised in both instances. Aspartic and glutamic acids are therefore the true metabolites of protein synthesis, and the formation of the amides, asparagine and glutamine, in the various experiments cited above, is a side-reaction. As we have seen, in the primary synthesis of protein these acids come directly from carbohydrate *via* the respiration process; in a resynthesis of protein from products of protein hydrolysis, the ammonia acts on deaminated products of the amino-acids, as well as on acids derived directly from carbohydrate in respiration.

'Acid' and 'Amide' Plants. This function of acid in protein metabolism has received confirmation by the discovery of Ruhland and Wetzel that in some of the strongly acid plants, such as Rhubarb and *Begonia semperflorens*, the acid is not formed solely in the respiration of carbohydrate but by the *deamination of amino-acids*, and instead of ammonia accumulating as amides (in 'amide' plants) it accumulates as ammonium salts of these organic acids. The mechanism of this deamination has already been discussed (p. 135), as also has the fact that in Rhubarb oxalic acid is the end-product of the deamination, malic acid appearing first (p. 123). In 'acid' plants, then, the decomposition of protein takes an alternative route to that already indicated for 'amide' plants, and may be summarised as follows:

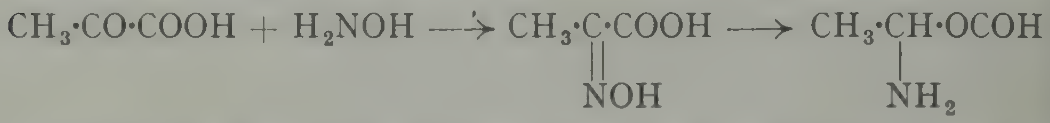
$$\begin{tikzcd} \text{Protein} \arrow[r, "\text{hydrolysis}"] & \text{amino-acids} \arrow[r, "\text{oxygen}", "\text{present}"'] & (\text{ammonia} + \text{organic acids}) \arrow[d] \\ & & \text{ammonium salts} \end{tikzcd}$$

This ammonia can then be used up in the synthesis of protein at the growing points, like the amides in the other type.

The Germinating Seed. Protein metabolism is simplified in the germinating seed in that there is no intake of nitrogen as nitrate or as ammonia, and therefore no synthesis of protein from new material. There is, however, hydrolysis of reserve protein, translocation of the soluble hydrolysis products to the growing tips of root and shoot, and resynthesis of these materials to protein. Investigation therefore centres round an examination of these hydrolysis products. In brief, they are found to consist of **amino-acids** and **amides.** In seedlings of *Leguminosæ*, asparagine occurs and can be induced to accumulate by etiolating the seedlings and so preventing the production of carbohydrates. The amount of asparagine derivable from the aspartic acid content of the reserve protein can be calculated, and in the *Leguminosæ* is quite small (about 2 per cent. of the total nitrogenous products in Lupin seed); whereas the asparagine actually formed in the plant is so much greater that it comprises the major portion of the hydrolysis products (73 per cent. in *Lupinus albus*). Similar results have been obtained with seedlings of many of the *Gramineæ*, *Pinus sylvestris*, *Picea excelsa*, *Tropæolum majus*, and *Helianthus annuus*. On the other hand, germinating seeds of the *Cruciferæ* and of *Ricinus* contain a high percentage of the amide glutamine. The transamination reaction itself can be examined by measuring transaminase activity (p. 290). In the Oat embryo, it was shown that increase in transaminase activity (measured by the action of the enzyme extract on glutamic acid + oxalacetic acid) was paralleled by an increase in the amounts of both soluble nitrogen and synthesised protein. The conclusion is therefore again that these amides are storage products for the ammonia formed by the decomposition of the reserve protein, if that ammonia cannot be used up at the growing points for the synthesis of new protein as rapidly as it is formed.

The Ripening Seed. In the ripening of seeds which store large amounts of protein, as in the *Leguminosæ*, we have the building up of reserve protein from soluble nitrogenous products translocated from other parts of the plant, especially from the leaves. The unripe seed is found to contain a high percentage of soluble nitrogenous compounds, while the ripe seed contains small amounts of the latter, and large amounts of protein. Experiments on ripening Peas show that the protein in the pod is hydrolysed to soluble nitrogenous products, which are translocated to the pea itself, where protein is synthesised. That is, the pod is performing the function of a reserve organ, and it is found that most of the soluble, nitrogenous material in the pod consists of asparagine, with much smaller amounts of various amino-acids.

The Function of Hydroxylamine. Hydroxylamine (NH_2OH) is the hydroxyl derivative of ammonia. As early as 1882, Meyer and Janny suggested that in the plant nitrate was reduced *via* nitrite to hydroxylamine, and that this compound was the **prime inorganic agent** introducing nitrogen into the organic metabolites in protein synthesis. Hydroxylamine is a common reagent for identifying aldehydes and ketones, forming oximes with them. Hence it can also react with the keto group of pyruvic acid forming the corresponding oxime or *iso*nitroso-derivative. This in turn can be reduced to the primary amine—in this case alanine—as follows:



$$CH_3{\cdot}CO{\cdot}COOH + H_2NOH \longrightarrow CH_3{\cdot}\underset{\substack{\| \\ NOH}}{C}{\cdot}COOH \longrightarrow CH_3{\cdot}\underset{\substack{| \\ NH_2}}{CH}{\cdot}OCOH$$

Experiments on higher plants have not yet demonstrated this possibility, but it has been shown to take place in *Fusaria*, and hydroxylamine is also formed by the symbiotic nitrogen-fixing bacteria in the root nodules of the *Leguminosæ*. The theory is that in the root nodules the atmospheric nitrogen is reduced by the bacteria to hydroxylamine; this condenses with oxalacetic acid (from the carboxylic acid cycle, p. 283) to form oximino-succinic acid, which is then reduced to aspartic acid.

The probable mechanisms of the syntheses of the cyclic nitrogen compounds in plants, *e.g.* the purines and pyrimidines (p. 169) and the alkaloids (p. 227), are discussed in the appropriate chapters.

CHAPTER XXVI

THE CHEMISTRY OF PLANT GROWTH

(It is recommended that Chapters I and II be re-read)

In Chapter I an attempt was made to classify the chemical compounds occurring in the plant, both with respect to their function in the plant and their chemical composition. The vital activities of the plant are concerned, *inter alia*, with the building up of these compounds, and with their decomposition and conversion into other products. In **germination** there is rapid cell-division, and the breaking-down of stored compounds to soluble products that are translocated to the growing points where the synthesis of new material occurs. At the same time, respiration takes place, and the seed loses in dry weight. When photosynthesis begins, the plant reaches the second stage in its development, namely, that of **growth** (in the restricted sense of increase in dry weight). Here two main types of processes coexist: (*a*) The synthesis of new materials, first the *photosynthesis* of carbohydrates with the absorption of solar energy, then from these the *synthesis* of proteins for new protoplasm and of the other substances, such as cellulose and fats, which help to build up the new tissue; and to these centres of growth there is the *translocation* of material. (*b*) The oxidative destruction of plant material in the *respiration* process, and the consequent liberation of energy. It is the balance of these two processes in the plant that results in growth. Finally, in the stage of **maturation** or ripening, photosynthesis declines and in all plants except evergreens ceases for the season; thus respiration predominates, and there is no further increase in the weight of the plant, although the seed itself gains in weight owing to the accumulation of storage material.

All the influences at work in the phenomenon of growth are not known, but there are at least three internal conditioning factors whose importance has been demonstrated, *viz.* water content, osmotic pressure, and p_{H} value.

Water Content. One of the main chemical reactions occurring n the plant is *hydrolysis*, and its reverse, *condensation* by removal of the elements of water. Instances are the conversion of starch nto sugar, and *vice versâ*, and of proteins into amino-acids. It is also significant that germination is accompanied by the absorption of water and is characterised by hydrolytic changes in the seed itself, although synthesis of new tissues of shoot and root takes place. On the other hand, maturation is accompanied by desiccation, and soluble translocated products are being condensed in the seed to give storage material. Changes in water content may also occur from cell to cell, and perhaps even within the cell if semi-permeable membranes are set up by such substances as lipins and nucleic acids, and these changes would exert a controlling influence on the reactions involving hydrolysis.

Osmotic Pressure. The cell-surface acts as a semi-permeable membrane, as is evidenced by the phenomenon of plasmolysis, and therefore can regulate the passage of both water and dissolved materials. There are therefore set up osmotic gradients between cells, which facilitate the movement of soluble substances, as, for example, in the germinating seed. In addition, within the cell there is evidence that the nucleoproteins (p. 169) and the phospholipins (p. 54) perform similar functions, partly by their amphoteric character and partly (in the phospholipins) through the presence of groups soluble both in water and fat solvents: each of these properties induces an orientation of the molecules, leading to the formation of surface films.

P_H. The importance of the hydrogen-ion concentration (usually measured by the p_H value), both of the cell-sap and of the protoplasm, has already been mentioned in connection with the reactions of substances with *amphoteric* properties, such as the proteins (p. 151). The effect of change of p_H value on the action of *enzymes* (p. 251) is also of fundamental importance in plant metabolism. The cell-sap, like other physiological fluids such as blood, is well buffered, because it contains in solution bicarbonates and phosphates of the alkali metals. The normal reaction of the **cell-sap** is slightly *acid* (pp. 115 and 217), while the normal reaction of the **protoplasm** is neutral or faintly *alkaline*. A deficiency of oxygen in the cell leads to an increase in acidity; this at first decreases respiration, but also activates the enzymes hydrolysing carbohydrates, and therefore gives a larger supply of easily respirable material to the plant. Here, then, an alteration in the p_H affects so many counteracting factors that the cell is protected against rapid changes.

Germination

The causes of the break in the dormancy of rest-period of seeds are many: some are external, and involve water supply, oxygen supply, the presence or absence of light, and a certain minimum temperature; while others are internal, and include after-ripening processes in the seed. There is also a sudden stimulus given to enzymatic activity, either by the removal of inhibitors or by an actual change in the enzyme, as from spermatolipase to blastolipase (p. 253).

Seeds can be divided into two groups, according to the principal storage material, *viz.* the **oil-storing** seeds which predominate (p. 34) and contain from 40 to 60 per cent. of oil, and the **starch-storing** seeds containing from 60–80 per cent. of starch. *Proteins* are present in all seeds, the higher content being usually found in the oil seeds (*cf.* Flax, 23 per cent. protein, Wheat, 10–13 per cent.); an exception is provided by the seeds of the *Leguminosæ*, which store starch and a large amount of protein (Peas, 23 per cent. protein and 54 per cent. carbohydrate; Soya Beans, 45 per cent. protein and 32 per cent. carbohydrate). Starch-storing seeds also contain small percentages of oil in the embryo (p. 45). The climate and supply of nutrient materials cause variations in the chemical composition of seeds. Climatic effects on Flax seeds have already been mentioned (p. 47), and experiments on Wheat and Barley have shown that a drier climate increases the protein content of Wheat, while a high nitrogen supply has the same effect on Barley. In germination, the hydrolysis and other transformations of these storage materials are catalysed by enzymes; the soluble products, namely, *carbohydrates* from fat and starch, and *amino-acids* and *amides* from protein, are translocated to the growing points for the synthesis of new tissue. The hydrolysis of starch is catalysed by diastase, which in the cereals is secreted particularly in the scutellum, and the first starch hydrolysed is that in the cells of the endosperm next the scutellum. Cytase is active in the hydrolysis of the hemicelluloses of the cell-walls (p. 94). The germinating seed is also the best source of the proteolytic enzymes (pp. 159 and 248), and of lipase. Certain other compounds occur in higher proportions in seeds than in any other tissue of the plant, particularly the *organic phosphorus compounds*, the **phospholipins, phytin,** and **nucleoproteins.** The rôle of phosphorus in plant nutrition appears to be associated with cell-division.

Growth

The normal period of growth begins with the commencement of photosynthesis by the chloroplasts; in addition, nitrogen in inorganic forms is absorbed from the soil for the synthesis of protein, and other ions such as phosphate and potassium are also absorbed. Growth itself is the *nett effect* of the synthetic processes, especially carbon assimilation, and the destructive process of respiration, and can be conveniently measured by the **percentage increase in dry**

weight. If the dry weight is plotted against time an S-shaped curve is obtained (fig. 9). During the 'grand' period of growth, the rate of increase follows the 'compound interest' law—that is, the increase is proportional to the amount present, until growth begins to fall off. (Not only does the whole plant give the S-shaped curve, but single organs such as leaves give the same type of curve.)

Growth is modified by various external conditions such as temperature and light. **Temperature** affects enzymatic activity and also the rate of chemical reactions, the effect of different temperatures on the oxidation of acids in fruit ripening (p. 122) and of climate on the composition of fats (p. 47) being examples. The effect of **light** is complex, photosynthesis being only one of the reactions affected by the intensity and quality of the light. For instance, although ultra-violet radiation appears unnecessary for plant growth (since several generations of plants can be grown in glass-houses, which cut off the short radiation), **blue** light is essential for normal vigorous growth, and its absence brings on conditions similar to those obtained when plants are grown in darkness or in light of very low intensity—namely, a rapid elongation of the stem and the production of very weak plants. Also the optimum period of illumination varies for different plants: for normal flowering and fruiting some plants require a short day, others a long one, and few plants can tolerate continuous illumination.

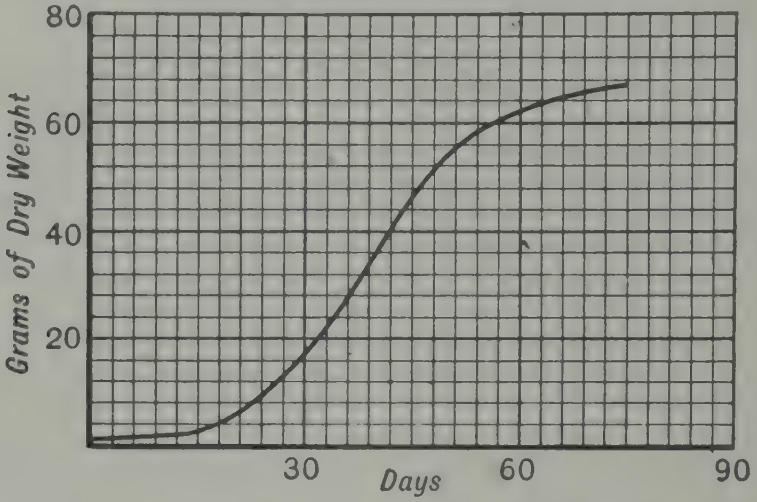


FIG. 9. The Increase in Dry Weight of Oats (Monnier).[1]

Plant Nutrients

The limiting effect of the concentration of **carbon dioxide** in the atmosphere on photosynthesis (p. 267), and therefore on plant growth, has been seen. The **water** supply is also a controlling factor, as large amounts of water are required by normal plants for transpiration, amounting in a year to about 300 times the weight of dry matter produced in the plant. In addition, the plant absorbs a large number of ions from the soil, including nitrogen as nitrate and as ammonia, phosphorus as phosphates, and potassium, magnesium, iron, calcium, sodium, silicon probably as silicate, manganese, and boron. All these elements (except nitrogen) can be detected in the ash (p. 1) from a plant. The question as to whether all these elements are 'essential' or not to plant growth is fraught with difficulty. In the use of culture solutions, for instance, practically any salt is toxic in moderate concentrations if fed alone, but if a mixture of several salts is supplied, no toxic effect is produced. Hence a **physiological balance** is necessary. Also the lack of one nutrient may be a limiting factor in the effect of other nutrients; while, in the plant, the stability of the protoplasm, which is colloidal, depends on the electrolytes. Again, some substances are required in large amounts by plants and are the basis of the manuring of crops, *e.g.* nitrogenous compounds, potassium salts, and phosphates. Others may be present in the soil in a form unavailable to plants (*e.g.* iron), or are lacking under special soil conditions (manganese), while still others are present in sufficient amounts in all soils, but have been shown to be necessary for plant growth in very low concentrations by carefully controlled water-culture (boron). How many elements belong to the latter class has not been determined, but it may be quite large, and all such elements are toxic in higher concentrations. Again, the effect of such nutrients on the plant is usually determined on the vegetative growth of the plant (increase in dry weight) or in the yield of fruit (as in cereals); but the effects on germination of the seeds and the resulting generations are rarely investigated, as such experiments are usually carried out from the point of view of crop yields only.

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[1] *Pub. Inst. Bot. Geneva,* 1905, Ser. 6, Fas. 111.

A short account of the function of the various elements in the metabolism of the plant follows.

Nitrogen. Nitrogen enters the plant as *ammonium salts* or as *nitrates*, except in the *Leguminosæ*, in which family the bacteria of the root nodules (*Bacillus radicicola*) enter into symbiotic relationship with the plant, and supply it with additional nitrogen which has been withdrawn from the atmosphere; another example is provided by plants with *Mycorhiza*, *e.g.* in the *Ericaceæ*, where the same type of symbiosis may occur. The stages in the absorption and assimilation of this nitrogen are not clearly understood. The amount of nitrogen given to and absorbed by a plant alters the **carbohydrate-nitrogen relationship** (C/N) in the tissues of the plant, and generally this change leads to two types of growth. A low ratio leads to vigorous vegetative growth, little flowering, and little fruit, whereas a high ratio in general gives a good development of the reproductive tissues, although a readily available supply of nitrogen is required at the time of flowering and fruit-setting, as practically all the nitrogen for the protoplasm of the fruit enters at that time, at least in Apples (p. 310). This is important commercially in fruit-growing; for instance, heavy nitrogenous manuring has to be avoided in the case of Apples and Tomatoes. This ratio also affects the storage life of fruit, since a high nitrogen content means a large amount of protoplasm, and therefore more vigorous respiration (p. 314). The relationship between *nitrogen* and *potassium* is also important, some fruit trees requiring relatively large amounts of nitrogenous manures, *e.g.* Plums, *Citrus* fruits, Blackcurrants, and Raspberries, whereas Apples, Pears, and Gooseberries require more potassium.

Potassium. Plants which store carbohydrate in seed, fruit, or underground organs react most to an increased supply of potassium salts. Potassium starvation is detected by pale-coloured leaves, which tend to die away at the tips, and in the cereals by shrunken grain. The chief function of potassium is to increase the **efficiency of carbon assimilation,** probably because an increase in potassium accelerates the absorption of carbon dioxide from the intercellular spaces by formation of bicarbonate. This increase in the photosynthetic process is most noticeable in dull seasons, and more positive effects have been obtained with the application of potassium manures in England and the North of France than in other countries with more sunshine. Potassium also counteracts the tendency to soft growth due to excess nitrogen, and plants receiving potassium manures are more resistant than others to fungal attacks. Plants differ greatly in the proportion of potassium present in the plant ash; ash from the Sugar-cane contains 88 per cent. of potassium and sodium calculated as the oxides, the remaining 12 per cent. being mainly oxides of calcium and magnesium. Contrast the ash of the Pea plant with only 28 per cent. alkalis, and 64 per cent. of the alkaline earths. Potassium is found particularly in the apices of the plant, and in rapidly growing regions such as young leaves. It appears to be withdrawn from older leaves to the stem, but accumulates in seeds and in underground stems storing carbohydrate.

Sodium. Sodium is found in all plants, but its rôle in plant nutrition is not well understood. In most cases it cannot replace potassium. Some plants contain much higher concentrations of sodium than others, especially those whose natural habitat is salt marshes (*e.g. Salicornia*), or the seashore (*e.g.* the Coconut Palm).

Phosphorus. Phosphorus is present in organic combination in plants in the nucleoproteins, in the phospholipins, and in phytin, while the phosphate ion is also present in the cell-sap. Phosphate is essential in the **respiration** process, as the first stage is the formation of hexose phosphate (p. 255). Phosphorus which is absorbed by the plant from the soil as the *phosphate ion* has two obvious effects on plant growth; in the early stages it promotes **root formation,** and is therefore always supplied to commercial root crops such as turnips and potatoes (contrast the effect of nitrogen on shoot growth), and secondly in the later stages it **hastens ripening.** Relatively large amounts of phosphorus are stored in seeds; here the organic compounds of phosphorus accumulate, especially in the nucleus, and such compounds appear to be necessary for mitotic cell-division.

Calcium. The effect of calcium compounds on the plant is twofold. All plants require a small amount of calcium, some more than others. In addition some plants, the 'calciphiles,' *e.g.* Mignonette, require a soil containing a high proportion of calcium carbonate; whereas others, the 'calcifuges,' *e.g.* Tea, cannot tolerate a chalk or limestone soil. This is probably due to the effect of the calcium on the p_H value of the soil solution, and therefore on the proportions of ions present. Some plants contain high percentages of calcium in the ash, especially in older tissues of the plant such as old leaves and the bark of trees; calcium also appears to be necessary for the growth of young tissues, especially roots. For instance, the highest calcium content of the outer leaves of Cabbage was found to be about 1 grm. per 100 grm. of fresh leaf, while a heart leaf contained only 0·026 grm. per 100 grm. In old tissues the main function of calcium is to neutralise plant acids, deposits of calcium oxalate being common (p. 115), but it also occurs as calcium pectate in leaves. Like silicon, and in contrast to potassium and phosphates, calcium remains in tissues such as old leaves, and is not withdrawn.

Magnesium. Magnesium is a constituent of the chlorophyll molecule, and is necessary for carboxylase activity. A few soils are deficient in soluble magnesium, and a type of *chlorosis* due to magnesium deficiency has been found in the Tobacco plant, in the Soya Bean, and in some fruit trees. Strawberries have been found to respond to manuring with magnesium salts also. Soluble magnesium, like phosphorus, migrates from the leaves when the latter age, and accumulates in seeds, mainly as magnesium phosphate. It is always present in much greater amount in oil seeds than in starch-storing seeds, but its special function in the former type is not known.

Silicon. Silicon is a characteristic constituent of the epidermis of the Grasses. The silicate ion is absorbed from the soil, and is dehydrated to silicon dioxide (silica) at the cuticular surface of the stems; silica appears also in the seed-coats. Its presence acts as a mechanical stiffening agent to the stems, and it also helps to prevent fungal invasion. To a smaller extent, silica is present in leaves, being returned to the ground with the old leaves and not moving back to the stem as does potassium.

Sulphur. Sulphur is a component of the protein molecule, but small amounts only are necessary, and these are usually present in sufficient amount in soils as sulphates. The *sulphate ion* travels up to the leaves, and like the nitrate ion is reduced in the synthesis of protein. Occasionally the addition of sulphates to the soil has proved beneficial, especially to crops rich in protein, such as the legumes (notably Alfalfa and Soya Beans). Chlorotic effects due to sulphur deficiency have been found in Tobacco and less severely on Apple trees. *Brassicas*, which contain a fair amount of sulphur, also respond to manuring with sulphates.

Iron. Iron is essential for the development and functioning of the iron prophyrin enzymes (p. 197) which take part in respiration. Its action in such organic combinations is that of an **oxidising-reducing agent,** as it can exist in the two forms, ferrous and ferric. Chlorosis—the yellowing of leaves—due to lack of iron is especially prevalent on chalk and limestone soils, for although these may contain plenty of iron, the soil solution is too alkaline to admit of sufficient ferric ions being present in solution (they are instead precipitated as the insoluble hydroxide).

Manganese. Most plants contain measurable amounts of manganese, the Gymnosperms being particularly rich in it. Manganese deficiency is another cause of chlorosis on chalk soils and has been found particularly on American soils in a variety of crops, *e.g.* Oats, Soya Beans, Spinach, Cabbage, and Tomatoes. Some of these plants exhibit definite pathological symptoms due to manganese deficiency, *e.g.* the 'speck' disease of Oats and the 'Pahala blight' of Sugar-cane. Manganese is essential for the functioning of some enzyme systems in which, like iron, it acts as an oxygen 'carrier.' Manganese occurs mostly in young leaves and in seeds, and is absent from old leaves and woody tissue.

Copper. Copper is present in all plants in small amounts, especially in actively growing tissues, and it accumulates in the seeds. Conjugated with protein, it forms some of the plant oxidases (p. 148). From experiments in culture solution, copper is necessary for flower and seed formation (in Barley), and the absence of copper has also been shown to result in poor growth of Tomatoes, Sunflowers, Beans, and Flax. An unusual case of chlorosis on *Citrus* trees in South Africa was found to respond to treatment with copper salts.

Boron. Boron is widely distributed in small amounts in plant tissues, especially in fruits. In a study of the assimilation of atmospheric nitrogen by symbiotic bacteria (*Bacillus radicicola*) in the root nodules of leguminous plants, Warington showed that minute traces of boron were necessary for the normal development of the meristematic tissues of both shoot and root; if boron was completely absent, the xylem degenerated in later stages of the plant's growth. In addition, symbiosis with the bacteria did not take place, the latter tending to become parasitic. As little as one part of borax per million in the culture solution was found to be sufficient for normal growth, while one part in two or three thousand was toxic. Boron is also necessary for the nutrition of non-leguminous plants such as *Citrus*, Potato, and Tobacco plants.

Other elements which have been regarded as 'essential' are aluminium, zinc, **iodine,** and **fluorine.** **Aluminium** may play a rôle similar to iron, and has been found in considerable quantities in leaves of Mulberry, Spinach, Radish, and Rhubarb. The beneficial effect of traces of **zinc** has been shown on cultures of the lower plant *Aspergillus niger*, and on soil cultures of Peas and Beans. Zinc occurs more particularly in the leaves and stems in plants, *e.g. Viola*, on soils rich in zinc, while zinc is also abundant in *Coniferæ.*

Plant Hormones and Auximones

Attempts have been made to find the cause of some of the **tropisms** which occur in plants, including those which cause a lateral bud to give rise to a vertical shoot when the leader is destroyed, and those which determine the rapid closing of the leaves and bending of the stems in *Mimosa* on the slightest bruising —the stimulus in the latter case being transmitted by the pulvini at the base of each petiole.

In animals, extracellular chemical substances have been discovered which move throughout the body and initiate various reactions. To these substances the name hormone has been given. Recent experiments by Wendt have shown that there is a **growth**

hormone produced by the growing points of the coleoptiles of the *Gramineæ*. If a cut tip is placed on a layer of agar gel, the latter can absorb the hormone; when the gel alone is then placed on the cut surface of the coleoptile, it will cause the latter to grow. Claims have been made that similar hormones exist in the hypocotyl of Lupin and Sunflower, and the action of **phototropism** and **geotropism** has been explained as an alteration in the amount and distribution of the growth hormone. Wendt, Kögl, and their collaborators isolated physiologically active material from malt and from Maize germ oil. This **auxin,** which also occurs in human urine, was shown to be **indole-β-acetic acid** (p. 226).

Auxins appear to be present in plant tissues in three forms: (*a*) free and active, *e.g.* indole-β-acetic acid isolated in crystalline form; (*b*) bound to protein, but still active; this type has been separated from Wheat germ by electrodialysis; (*c*) bound to protein in an inactive form. This type has been isolated from Spinach leaves; it constitutes seventy-five per cent. of the total protein in the cytoplasm, and contains all the potential auxin activity. Auxin appears to affect the *rate* and *direction* of some of the *enzyme systems* in plant metabolism. Its precursor in the plant may be tryptophan. Supplied artificially, indole-β-acetic acid can induce dormancy, and inhibit bud formation. It can also promote root formation, and prevent leaf and fruit drop. The effect of ethylene in fruit ripening (p. 316) appears to be due to the liberation of bound auxin.

Various compounds chemically related to indole-β-acetic acid have been synthesised and found to exhibit auxin activity. α-Naphthalene-acetic acid is frequently used to replace indole-β-acetic acid in inducing the rooting of cuttings, in stimulating artificial pollination, and (in very dilute sprays) in preventing fruit drop, especially in Apples and Pears. 2, 4-Dichlorophenoxy-acetic acid has such a stimulating effect on top growth that it is used in weed control—the roots cannot grow and assimilate sufficient nutrients to keep up with the top growth, and the plant starves.

Traumatins are growth-promoting substances extracted from wounded plant tissue (ground or heated). They induce cell division and extension in the parenchymatous cells of the Bean pod mesocarp. A 12-carbon unsaturated dicarboxylic acid, **traumatic acid** ($HOOC{\cdot}(CH_2)_8{\cdot}CH{:}CH{\cdot}COOH$), was isolated. English synthesised it and showed that it was effective as a 'wound hormone' in inducing periderm formation in discs of Potato tuber, and in replacing the juice of Tomatoes as an inhibitor of the germination of the seeds.

Although normal growth is possible in purely inorganic media, the absorption of small amounts of organic matter from the soil or from culture solutions, resulting in an increase in plant growth, has been postulated by several workers. Bottomley termed such substances **auximones.** Ashby has shown that minute amounts (less than 0·02 per cent. of the mineral matter in the culture solution) of organic material from horse dung increased the growth rate of the aquatic plant, *Lemna minor*, to a marked degree. An increase in the area of the fronds and in the number of chloroplasts per frond was found, with the result that the photosynthetic efficiency was increased, and therefore also the growth rate. It is not unlikely that in such cases the plant is absorbing more readily from the soil or culture solution what it can, if necessary, synthesise by itself, *viz.* the traces of compounds necessary to form the various enzyme systems. It can be shown that thiamine (vitamin B_1), riboflavin (B_2), pyridoxine (B_6), and nicotinic acid or nicotinamide (B complex) can all affect plant growth; vitamin B_1, for instance, is used to promote rapid root growth in transplanting seedlings. However, the greatest effects of these substances are shown on the excised roots of higher plants, and the intact plant probably can synthesise them sufficiently rapidly for its normal needs.

CHAPTER XXVII

MATURATION, FRUIT RIPENING, AND STORAGE. THE CHEMICAL EFFECTS OF COLD AND FROST ON PLANTS

MATURATION

A DISTINCTION must be drawn between the ripening seed, which is accumulating food reserves for the new plant, and the maturation of the fruit. Another form of 'ripening' is the storage of food materials in roots, tubers, and woody stems. The chemical composition of **seeds** has already been noted in connection with their germination (p. 297), and the synthesis of fat in seeds from carbohydrates has also been discussed (p. 46). In starch-storing seeds, the starch is also formed *in situ* by the condensation of soluble carbohydrates, which move from their seat of synthesis in the leaves to the seeds. Similarly in the *Leguminosæ*, the protein is built up in the seed from soluble translocation products, the amide asparagine predominating, along with smaller amounts of amino-acids. Here the seeds are enclosed in pods, and the latter form temporary storage centres in which protein is hydrolysed; the resultant products migrate to the seed, where protein is again synthesised.

In **underground organs,** the principal storage materials are starch, inulin, and the sugars—sometimes sucrose alone, more often mixtures of glucose, fructose, and sucrose. The translocation material which goes to the synthesis of these carbohydrates appears in most cases to consist of glucose and fructose. Occasionally fat is stored in such organs, and some contain a fair amount of soluble nitrogeneous products when removed from the ground, *e.g.* Mangolds, but on storage these are converted into protein.

The **Carbohydrate-Nitrogen relationship** (C/N) is of importance in the quality and storage life of both seeds and fruits. The ratio depends not only on the supply of nitrogen, but also on the soil type and the climate. For instance, in the *Gramineæ*, light soils in Britain give a higher C/N ratio than heavy soils, as the former are conducive to early ripening and hence to the complete assemblage of the carbohydrate as starch in the seed. On heavy soils, and in northern and western districts where ripening is less complete, the straw still contains soluble carbohydrates. The effect of climate on the C/N ratio of Wheat grain is very marked. When most of the rainfall occurs in the growth season (steppe climate), the grain has a high nitrogen content and therefore a low C/N ratio, whereas with the maximum rainfall in winter there is less nitrogen and a higher C/N ratio. Similar results have been observed in the ripening of fruits, especially Apples.

Fruit Ripening

Fruit ripening involves the maturation of both the seed and the enclosing tissue, and the latter has been most studied because of its commercial importance in many cases as foodstuffs. The most comprehensive investigation on the chemical changes occurring in the ripening of fruit and its storage under different conditions has been done in Britain under the auspices of the Food Investigation Board. Apples were the fruit most completely examined, but analyses of other fruits have also been made.

Chemical Composition and Growth of Apples. Apples consist of (i) **cell-wall materials,** *viz.* cellulose, hemicelluloses, and pectic substances; (ii) **protoplasmic protein**; (iii) **starch**; (iv) **sugars,** *viz.* sucrose, fructose, and glucose; (v) **acids,** especially malic acid. The major changes in the apple are connected with alterations in the concentration of these substances. The composition of most fruits is similar, but the ratio of the various constituents differs widely. In addition, fruits contain ions in solution, salts, and small amounts of organic substances such as esters, tannins, and glycosides.

The **acid content** of apples differs markedly among the varieties, and this difference is reflected in other chemical changes taking place in the fruit. An approximate division can be made between **high acid varieties,** of which Bramley's Seedling is the example investigated, and low or **sub-acid varieties,** such as Worcester Pearmain. Small variations in the acid content of either group of the same physiological age do occur; like the variations in sugar content, they appear to depend mostly on the conditions throughout the growth period, *i.e.* the acid content for one variety changes with soil type, and with climatic conditions, especially temperature. For instance, Bramley's Seedling apples from the colder parts of England have a higher acid and lower sugar content than those from warmer districts, and in the same orchard a similar difference can be found between cold and warm growth-seasons.

Nitrogen content, unlike acid content, is determined chiefly by the soil, and is approximately constant from year to year for different varieties in the same orchard, varying markedly for the same variety in orchards with different soils. Increasing the nitrogen supply by manuring influences the yield and size of the fruit, but does not always affect the percentage of nitrogen in the fruit. This result is of importance in a consideration of the keeping qualities of apples, as for all varieties high nitrogen content denotes a large amount of protoplasm; this, in turn, is linked with a high respiration rate, and therefore a more rapid exhaustion of respirable material. Fig. 10 shows the changes in concentration of the various constituents of the apple from petal fall to maturity, and has been termed by Archbold the **Growth Phase.**

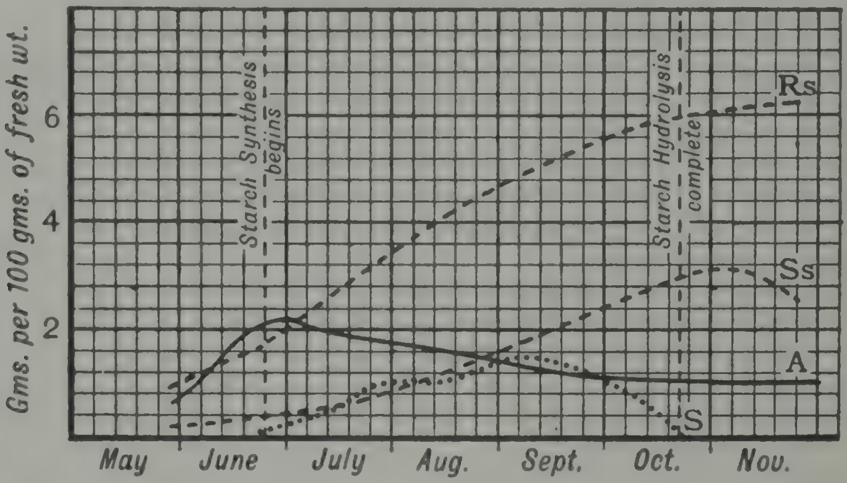

FIG. 10. The Concentrations of the Constituents of Bramley's Seedling Apples during Growth, May to November 1930 (after Archbold).[1]

Rs = Reducing Sugars; Ss = Sucrose; S = Starch; A = Acid
Grower's Gathering Date, 21st October

The growth phase can be divided into four stages as follows:—

Stage I is characterised by the formation of cell-wall material, the intake of nitrogen, a rapid increase of the size of the fruit, and a slow accumulation of sugars and acid. This lasts from the end of May to the middle or end of June (about 4 weeks for Bramley's Seedling and 3 for Worcester Pearmain), and is terminated by the appearance of starch. The simultaneous formation of cell-wall material and organic acids may be due to their derivation, as suggested by Archbold, from the carbohydrate, probably a sugar, supplied to the fruit. The cell-wall materials would be condensation and oxidation products, with the acids as oxidation products probably formed in respiration. The fact that sub-acid apples accumulate a high percentage of cell-wall material, while high acid apples have a low content, supports the contention.

Stage II is the period of *starch synthesis*, lasting for 5–6 weeks in Worcester Pearmain (middle of June to end of July) and for 8–10 weeks in Bramley's Seedling (middle of June to end of August). This is the period of maximum synthetic activity, with an increasing rate of accumulation of total solids, including both sucrose and the hexoses, but especially of fructose. At the beginning of Stage I the concentration of glucose is higher than that of fructose (*e.g.* 0·32 per cent. of the fresh weight is fructose, and 0·86 per cent. glucose, in one analysis of Worcester Pearmain); but during Stage II the rate of increase of fructose rises rapidly, while that of glucose remains almost constant, giving the large excess of fructose over glucose (*e.g.* 6·39 per cent. fructose and 1·27 per cent. glucose) which is characteristic of the mature apple. The maximum value of acid is reached just after the appearance of starch; in the same year and from the same orchard, Bramley's Seedling contained 2 per cent. at the maximum, whereas the Worcester Pearmain content was 1·3 per cent. In both types, acid content then falls at a decreasing rate throughout the rest of the growing period.

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[1] *Ann. Bot.*, 1932, 46, 418.

Stage III is the period of *starch hydrolysis* and lasts from the end of July till early September for Worcester Pearmain, and from the middle or end of August to the middle of October for Bramley's Seedling. During Stages II and III alike, there is a continuous increase in total sugars, especially in sucrose and fructose, and the *maximum for total sugars* (and also for total dry weight) occurs just after the disappearance of starch, *i.e.* at the beginning of Stage IV. This change in sugar concentration is not entirely or even mainly due to the starch hydrolysis; there is in addition a large intake of sugars during this period.

Stage IV is the period of maturation, and may last only a few weeks. The maxima for total solids and sugar concentration occur at the beginning of this stage, and this is the normal gathering time for apples, especially Bramley's Seedling. Worcester Pearmains, however, are often gathered in commercial practice before starch hydrolysis is complete. If the fruit is kept on the tree, the sugar concentration is found to remain constant for some time and then to decrease slowly, owing mainly to a falling off in the sucrose concentration, while fructose and glucose remain constant.

Fruit still on the tree now enters a completely new phase, *viz.* the **Senescence Phase,** and the transition is marked by a change in the *respiratory rate.* During the growth phase, the respiratory rate, which is high at fruit setting, gradually decreases; as it almost parallels the acid concentration curve, it would appear that during this phase respiration is governed by the concentration of acid present. On senescence, however, there is an abrupt change in the respiratory rate, which may increase by 50 or even 150 per cent., and this has been termed the **climacteric phenomenon** by Kidd and West (fig. 11). It has been suggested that the climacteric is due to a change in the state of the protoplasm, leading eventually to its auto-destruction. Both Pear and Banana fruits exhibit this climacteric phenomenon between growth and senescence.

During senescence, the respiratory rate is no longer parallel to the change in acidity; it decreases, finally to zero, and the 'death' of the apple supervenes, accompanied by fungal attack or by physiological breakdown. This climacteric is not necessarily associated with the gathering or fall of the apple, but whenever the fruit is dissociated from the tree, it will undergo chemical changes as a detached organ; these changes are therefore only degradation reactions. The time of picking the fruit with regard to its physiological age, *i.e.* in relation to its stage of maturity and the climacteric, does, however, influence its behaviour on storage.

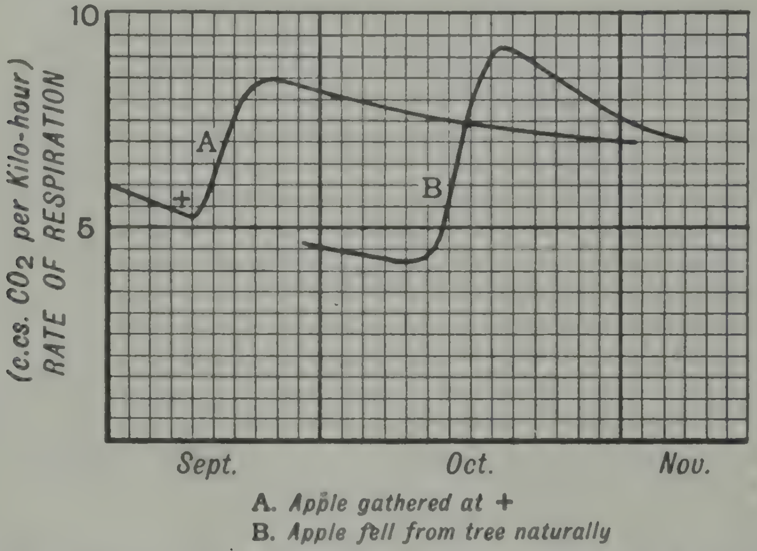

FIG. 11. The Climacteric Phenomenon in the Respiration Rate of Apples (Kidd and West [1]).

Fruit Storage

On storage, there is a continuous loss of dry weight through respiratory activity, due to loss of total sugars and acid; and sound apples, after starch hydrolysis is complete, consume sugars at a rate which either remains constant or changes very slightly until breakdown ensues. The amounts of cellulose, hemicelluloses, and pectic substances do not change much on storage, although there is conversion of insoluble protopectin into soluble pectin.

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[1] *Report of Food Investigation Board*, 1924, 31.

A soft over-ripe condition occurs simultaneously with the disappearance of the middle lamella pectic substance.

The length of the storage life of apples depends (*a*) on the respiratory rate, *i.e.* on the *demand* for respirable material; and (*b*) on the *supply* of respirable material available. (*a*) The respiratory rate depends directly on the **nitrogen content** and also on the **time of picking.** Apples picked near the climacteric maintain their high respiration rate on storage. With regard to the nitrogen content, for any *one variety* from different soils and therefore containing varying amounts of nitrogen, the *loss of total dry weight per unit of nitrogen* is practically constant. With different varieties, some are characterised by relatively high losses per unit of nitrogen, *e.g.* Worcester Pearmain. (*b*) The respirable material appears to differ with the period of storage life, which has therefore been divided into three stages. The changes in concentration of the various substances during these stages is shown in fig 12.

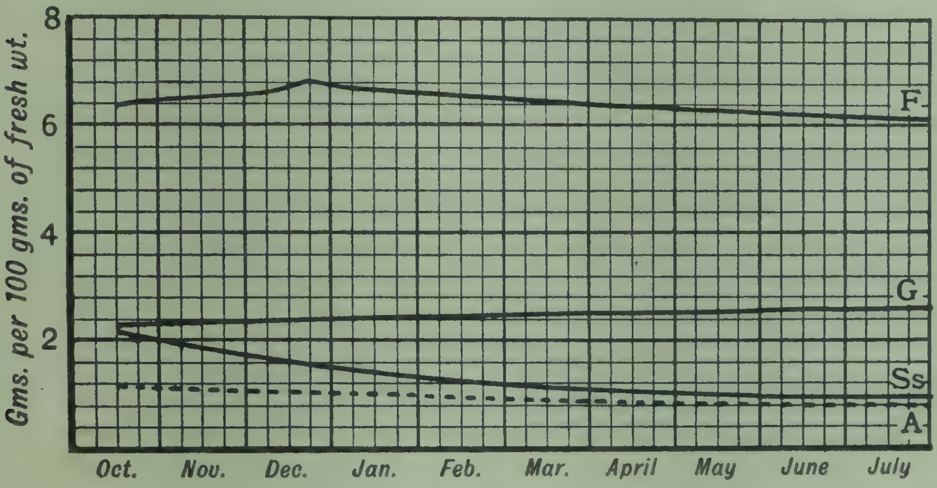

FIG. 12. The Concentration of the Constituents of Bramley's Seedling Apples during Storage at 1° C. (after Archbold).

F = Fructose; G = Glucose; Ss = Sucrose; A = Acid. There was no starch present

Stage I occurs until all the starch has been hydrolysed, and the products of this hydrolysis, shown by an increase in reducing sugars, are the principal substrate for respiration. This stage will, of course, be absent in apples which have not been gathered until starch hydrolysis is practically complete, *e.g.* Bramley's Seedling.

Stage II starts with total sugars at their maximum, and the respirable material is now supplied by the inversion of sucrose. During this period the rate of sucrose inversion exceeds that of sugar oxidation, and therefore there is a rise in reducing sugars which is due almost entirely to fructose. Either glucose, or fructose, or both, are respired; but as in some cases the initial rate of sucrose inversion is more than double the rate of loss of total sugars by respiration, there must also be conversion of glucose into fructose. Finally, after from six to eight months of storage life, the reducing sugars reach a maximum due mainly to fructose. Low temperature storage appears to prolong this stage.

Stage III follows this maximum, when there is a decrease in the concentration of reducing sugars as well as in sucrose. The respirable material now consists not only of the products of sucrose inversion, but also of stable reducing sugars, especially fructose. With sound apples, the rate of loss of sugars is somewhat more rapid in Stage III than in II. In varieties which are susceptible to *internal breakdown* (*i.e.* physiological disintegration of the tissue), this usually sets in at the beginning of Stage III, but it is not a necessary concomitant. If breakdown does occur, there is a distinct rise in the rate of loss of sugars and in loss of acid, which is otherwise at a low constant value. Apples which have reached Stage III have little commercial value.

Pears and **bananas** show similar changes on ripening and storage. With bananas, which contain a high percentage of starch, Stage I is much prolonged.

The influence on storage life of both the demand for and the supply of respirable material takes two forms in the case of apples, depending on whether they are high acid or sub-acid varieties. In *high acid* apples such as Bramley's Seedling, there is an *inverse* relationship between **sucrose** content and **nitrogen** content, whereas in *sub-acid* apples such as Worcester Pearmain the relationship is *direct*. Hence a low nitrogen content, and the consequent high sucrose content in the first type, ensure both a low respiratory rate and also a large supply of respirable material, and therefore good keeping qualities. With a sub-acid variety, however, a low nitrogen content will give a low respiratory rate, but there will also be a smaller supply of respirable material, *viz.* sucrose. The variation in keeping quality of *one* variety grown on different soils is shown in the following instance. Defining 'mean storage life' as the interval before 50 per cent. loss of the fruit occurs, Bramley's Seedling from a *silt soil* (Lincolnshire) had a storage life of 252 days, while from *fenland* (Cambridgeshire) the same variety had a storage life of only 184 days, both at 8° C. The first type had low nitrogen content, while the second had high nitrogen.

The *respiratory rate* changes with **temperature,** and is slowed down in 'cold' (1° C.), as compared with 'common' storage. But against this advantage is the fact that some varieties are liable to a physiological breakdown at low temperatures, and therefore different varieties show different *optimum temperatures* for maximum storage life, *e.g.* −1° C. for Newton Wonder (resistant), +3° C. for Bramley's Seedling (moderately susceptible), and 4·5° C. for King Pippin (very susceptible). Many other fruits show similar low temperature breakdown, *e.g.* oranges, plums, some varieties of peach, and pineapples. Another type of breakdown to which some varieties of apple are susceptible occurs at *ordinary temperatures*, and can therefore be delayed by cold storage. As it is shown especially by the Jonathan variety, it is often referred to as 'Jonathan breakdown.'

Both 'cold' and 'common' storage referred to above are in air. Another method of lowering the respiratory rate, which has proved a commercial success, is known as 'gas' storage. This consists in storing the apples in mixtures of nitrogen, oxygen, and carbon dioxide, in which the oxygen content is lower, and the carbon dioxide content higher, than in ordinary air. 'Gas' storage, within the temperature range of susceptibility of the variety in question to low temperature internal breakdown, is worse than air storage of either kind; but at temperatures above this critical temperature it is the best form of storage. For Bramley's Seedling, 10 per cent. of oxygen and 10 per cent. of carbon dioxide at a temperature of 4·5° C. was found to give the longest storage life. Higher concentrations of carbon dioxide bring about anaerobic respiration with the accumulation of ethyl alcohol and acetaldehyde (p. 22); the latter is especially toxic and causes a breakdown known as 'brown heart.'

Acetaldehyde is developed in small amounts in the normal ripening of *apples* and *pears*, especially when the fruit is softening rapidly. Small concentrations of acetaldehyde in the air of the storage chamber have actually been found to inhibit fungal growth of a number of fruits—*e.g.* oranges, grapes, cherries, and plums—and any acetaldehyde which is absorbed by the fruit is removed by oxidation in the respiratory process. That this occurs has been shown in the storage of *oranges*, the excess of carbon dioxide having been found to balance the small amount of acetaldehyde absorbed, according to the equation:

$$2CH_3{\cdot}CHO + 5O_2 \longrightarrow 4CO_2 + 4H_2O$$

On the other hand, the accumulation of volatile products from stored fruits must be avoided as it causes a discoloration of the skin of the fruit, termed a 'superficial scald.' The purpose of oiled paper wrappings in commerce is to absorb these products and prevent such damage.

Pears (Conference variety) can be stored at 1° C. with a low concentration of oxygen (1–2 per cent.), and the respiratory rate is so depressed that they can be kept under such 'gas storage' conditions for several months.

Artificial Ripening

The artificial ripening of fruit after harvesting has been used for centuries by the Chinese, who ripen hard *pears* by placing them in closed chambers in which incense is burned. Again, it was found that the change from green to yellow in the skin of *lemons* could be accomplished by storing the fruit in air-tight rooms in which kerosene stoves were used or into which the exhaust gases from a petrol engine were admitted. Denny showed that **ethylene,** the simple unsaturated hydrocarbon, $CH_2{=}CH_2$, was the effective agent; and ethylene is now used commercially, especially in the United States, in the ripening of oranges, bananas, tomatoes, pineapples, and dates, the best concentration of the gas being one in one thousand. The effect of ethylene is physiological rather than directly chemical; it probably affects the enzymes which control the many changes taking place in fruit ripening. As the change of colour in normal ripening appears to be caused partly by the liberation of volatile compounds from the fruit itself, the occurrence of coloration in fruit such as lemons and tomatoes is not surprising. Acid and carbohydrate changes appear to follow the normal course; for instance, treatment with ethylene increases the sugar content of tomatoes, and converts starch into sugar in bananas. Similarly, it has been found that the vapours of many chemicals, such as **ethylene dichloride,** $C_2H_4Cl_2$, **ethylene chlorhydrin,** $ClCH_2{\cdot}CH_2OH$, and **acetaldehyde,** $CH_3{\cdot}CHO$, break the dormancy of Potato tubers, which will sprout from two to six weeks earlier, and of buds of woody twigs such as Apple, Plum, Lilac, etc. Apple cuttings have been made to bud two months earlier by such treatment. In high concentrations, all these substances are toxic to plants; it is thus possible that low concentrations may cause slight injury to the cell-wall tissue, thereby liberating enzymes or perhaps also stimulating growth hormones (p. 306). A marked increase in catalase and diastase activity is always found in artificial ripening.

THE EFFECT OF COLD AND FROST ON PLANTS

Alpine plants can tolerate very low temperatures; it is also well known that many plants die off if exposed suddenly to low temperatures, although if they are gradually exposed to cooler conditions they can survive. This latter process is called **hardening.** In plants which are readily killed by frost, *e.g.* Dahlias, Roses, death is due to **desiccation,** as these plants are unable to absorb water from the soil at low temperatures, while the loss of water by transpiration continues. When less susceptible plants die on sudden exposure to low temperatures it has been shown that **ice crystals** begin to form in the *intercellular spaces*, and water is gradually drawn from the cells for the growth of these crystals. With a short exposure, such plants may recover on thawing; but in many cases, owing to the excessive desiccation of the protoplasm in the cells, it is found that the cells are dead on thawing, although the cell-walls may not be ruptured. What usually happens is that, in vacuolated cells, the loss of water by the vacuole and the contraction of the protoplasm are both so great that the *plasma sac is ruptured*. This is borne out by the fact that cells with large vacuoles are readily killed by frost, whereas cells entirely filled with protoplasm are more resistant. An intermediate effect occurs in herbaceous plants, *e.g.* Cabbage, which exhibit transparent spots or 'injected areas' composed of air-free intercellular spaces after the ice in them has melted and the water so formed has been absorbed by the cells. Sometimes also a type of tumour growth occurs on these spots in Cabbage, Lettuce, and certain other plants on thawing. Thus, some plants cannot survive the formation of ice crystals in the tissues, while others can, especially after being hardened. One factor which assists plants is the presence of a waxy bloom on the leaves. On a plant containing no bloom, moisture, or even snow, collecting on the leaves will penetrate to the intercellular spaces and take part there in the growth of the ice crystals; whereas a bloom on the plant, *e.g.* as on Cabbage leaves, prevents any connection between the external water and the intercellular spaces, and the water in the latter can remain under-cooled without ice-crystal formation setting in. The effect of low temperatures on the cell depends on at least two factors: (i) the amount of water removed from the cells; and (ii) the ability of the protoplasm to withstand freezing. This latter factor involves the peculiarities of the composition and colloidal stability of the protoplasm of each plant. For instance, if the extracted sap of plants is subjected to increasingly low temperatures till precipitation of protein takes place, this occurs first in the susceptible plants, *e.g.* – 3° C. for Bryony, and at much lower temperatures for resistant plants, *e.g.* – 15° C. for winter Rye, and – 40° C. for Pine needles.

Hardening. The process of hardening has been shown to be of two kinds: (i) hardening above 0° C., when chemical changes take place, especially the *accumulation of soluble carbohydrates;*

(ii) hardening below 0° C., when *dehydration* is the important factor. A striking experiment of the former type is due to Harvey. One set of Cabbages was hardened by exposure to 3° C. for 5 days; this set and an untreated set were then exposed to − 3° C. for 30 minutes. Both sets were frozen stiff; the latter was killed, while the former recovered on thawing.

(i) A change in temperature will not necessarily affect all reactions such as respiration, enzymatic hydrolysis, etc., to the same extent. One of the chief effects of temperatures from 6° C. down to 0° C. is to prevent the formation of starch in plants in which photosynthesis takes place at such temperatures, and also to cause the conversion of starch already present in the tissues into sugars, especially hexoses. The best-known example is the conversion of some of the starch in Potato tubers into sugars at low temperatures. Similarly in winter, Onion bulbs contain less sucrose and more reducing sugars than in summer, while Lidforss showed that the leaves of hardy plants such as *Veronica* and *Viola* contain sugars only in winter. More abnormal cases of sugar production at low temperatures are of *galactose* on Ivy berries and of *fructose* on Tomatoes. The formation of molecules which are present in true solution in the cell will *lower the freezing-point of the cell-sap*, and this is effected by the change from starch (insoluble) to sugars (soluble), or even from sucrose (1 mol.) to hexoses (2 mols.). That this is not the complete explanation of hardening is shown by the fact that some plants having high concentrations of soluble carbohydrates are not frost-resistant, *e.g.* Beetroot. Another chemical change taking place is the *partial hydrolysis of protein* to products less easily precipitated. Again, *fats* are often formed from stored carbohydrates under low temperature conditions, especially in trees. The effect of fats is to lower the surface tension of the cell-sap, which is equivalent to lowering the amount of 'free water' in the cell; so that, with the same amount of soluble substances present, the concentration is greater and therefore the freezing-point is lowered. The killing of plants by a sudden, but relatively mild, frost in spring when they have survived much lower temperatures in the winter is also explicable, for in winter all these chemical changes have taken place and the plant can resist low temperatures; whereas after the warm weather in the spring the reverse changes have occurred, and the plant can offer no resistance to a sudden drop in temperature.

(ii) The main change in plants on hardening below 0° C. is *dehydration*. Plants in a wilted state are more resistant to frost than turgescent ones; hardy varieties of Wheat, for instance, normally contain less water than varieties grown in warmer latitudes. In some cases it is not the total amount of water that is different: for example, in resistant plants or hardy varieties, the plant colloids can absorb more water, leaving less 'free water' to dissolve the salts and sugars present; so that again a higher concentration is obtained.

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